

Crying Wolf in the Dark

THE most serious deficiency of the attempt by the Select Committee on Science and Technology (see page 140) to contribute to the understanding and solution of an important social problem is that the committee talks about what it calls a population policy without attempting to define the ingredients that might constitute such a policy. Instead, the committee provides an aimless echo of evidence put to it in 1970 about the likelihood that the population of Britain will continue to increase and a sombre but familiar recitation of some of the consequences that may stem from such a trend. Gone is the spirit of the 1930s, when in Britain, as in France, it seemed proper to hope for at least a sufficient increase in population to provide some sense of national progress. Instead, the committee has taken the more fashionable view that more means worse (*pace* Mr Kingsley Amis). For all its gloom, however, the committee has nothing better to suggest than that the practical problems of continuing analysis and appraisal should be carried out by yet another office of government, presumably an amalgam of the Registrar-General's department with other interested organizations. The committee, which collectively has plenty of experience of the pitfalls of modern government, should know that no useful purpose is served by attempting to dignify and solemnize an important problem by saying that those in charge of it should be responsible directly to the Prime Minister.

Untackled questions abound. In the committee's declaration, the reasons for fearing the growth of population in Britain are exceedingly hard to discern. The report includes a statement that it is necessary "to act twenty years in advance in order to influence a trend in population figures" but this is hardly worth the italics lavished on it. Does not every schoolchild expect that the time scale of demographic change is likely to be comparable with the time taken for newborn girl babies to become fecund? Equally, however, it is plain that attempts to predict the rate of growth of population at times more distant than two decades or thereabouts are likely to be frustrated by changes in marriage patterns, fertility patterns and the like. The committee members who no doubt shake their heads in bewilderment about the habits of the contemporary young should perhaps consider whether they are confident that the reproductive patterns of their own generation are likely to be applicable when those now infant are adult. This point was put forcefully to the committee when taking evidence by Professor D. V. Glass who also showed how frail are the methods at present used for predicting future trends of population. The hazards of these predictions are perhaps most easily appreciated by recalling that the population increase between now and 2000 will be the difference between two very large numbers representing total births and total deaths and departures between now and then. Solomon would be taxed.

Statistical techniques undoubtedly leave much to be desired, and outsiders must ask whether it is really necessary that forward projections of population should be attempted only when comprehensive figures are available

from the records of official bodies such as the Registrar-General's office, the registrations of births and deaths and the census every ten years. If the problem of the growth of population is important, is there not a case for using sampling techniques to yield a quicker result? At present, however, the greatest uncertainties are not arithmetical but sociological. Thus there seems to have been for several years a beneficent competition between the tendency for the mortality of young women to decrease and the tendency for fertility to decrease, especially among older women. The most fertile group of all, women in their early twenties, tended to produce 178 children per 1,000 women per year in 1965 but only 160 children per 1,000 women per year in 1968. In the same interval of time, however, the fertility of women in their early thirties had decreased from 101 children per 1,000 women per year to 88 children per 1,000 per year. If there is indeed an urgent need to understand the future trends of the British population, is there not a serious case for asking for a better understanding of these tendencies? Is it fashion or contraception that has helped women to cram their childbearing into a shorter time span? Why is fertility decreasing even among younger women? In short, one of the most important tasks, to which the Select Committee might at least have paid the courtesy of a mention, is for the kind of social research which could in the years ahead define the forces by which future demographic patterns will be moulded. One of the most depressing bits of evidence uncovered by the committee in its investigation was the declaration by Dr Jeremy Mitchell, secretary of the Social Science Research Council, that applications for support in population research had been few in number and poor in quality. Is there any reason to expect that a new arm of the bureaucracy reporting direct to the Prime Minister will help to remedy this state of affairs?

The Select Committee's failure to grapple with the demography of its problem is, however, dwarfed by the emptiness of its qualitative judgments about the consequences of population growth. To judge from its report, the committee has served chiefly as a reflector of prejudices about the discomforts of change. The committee repeats, for example, the view that "overcrowding in a rising population can have an adverse effect on human faculties and on the quality of life in general". In practice, unhappily, even a close reading of the evidence reveals that the committee is quite bereft of what ordinary mortals would call evidence—the nearest the committee seems to have come to a proof that overcrowding as such brings trouble was a denial by Sir George Godber, Chief Medical Officer at the Ministry of Health and Social Security, that the results of Colquhoun's experiments with overcrowded rats are applicable to human populations. The committee offers no comment on Sir Solly Zuckerman's telling question as to why people seem to choose to congregate in cities, when even in Britain centrifugal tendencies might have driven them to populate the depopulated highlands of Scotland and elsewhere. Instead, the Select Committee on Science and



Technology has lent its reputation, diminished though it may be by the publication of its report, to the notion that the ideal state of affairs is one in which the population is spread as uniformly as possible over the surface of the country. Not merely is this shallow belief a poor pretence at a population policy, but it constitutes an entirely unnecessary piece of prejudice about the nature of the good society that is probably as wrong as it could be.

In circumstances like these, it is clearly difficult to know whether a population policy is a realistic concept. It may well be that attempts to change the course of events must be planned not over twenty years, as the committee suggests, but over much longer periods. In the long run, no doubt, the general climate of opinion about the desirability or otherwise of a growth or contraction of the population is probably the most effective determinant of change, but even so it is surprising that the committee has entirely neglected to examine the role that might be played by strictly political devices such as the incidence of taxation (or social benefits) on families of different sizes. Is it not paradoxical, after all, that the committee members who now wring their hands in discontent about the prospect of an extra thirteen million people in Britain should also unthinkingly vote each year for legislation that provides relief from taxation with each extra child. Although the system is not so tightly geared that families can hope to make a profit from taxation by overbreeding, the patent possibility of pushing events the other way by means of adjustments to the provisions for taxation seems to have been entirely neglected. So naive is this whole section of the committee's discussion that it must be wondered whether it has not deliberately decided to steer away from politically painful questions.

On the assumption that steps can be taken to regulate the size of the country's population, what criteria should be used for choosing directions in which to steer? Surprisingly enough, most discussions of the problems of population are based simply on estimates of total numbers. It is, however, well known that different sections of the population make different kinds of demands on resources and the environment, and contribute in different ways to the health of society as a whole. Children, for example, need schools. Women contribute less fully to economic prosperity than do men, partly because of the illiberality with which they are treated but also because they are necessarily more directly involved with making the population grow. Old people

are economically unproductive but, for the sake of the social conscience, their continued increase is to be welcomed. So does it not follow that if the Select Committee's proposed Special Office (directly responsible to the Prime Minister) were in being, would it not be necessary to balance the need to have enough people in the labour force to sustain the GNP against the cost and difficulty of providing for their demographic companions, old and young? In countries such as Britain, it might even be necessary to aim not at predetermined numbers in the total population nor even at predetermined numbers of working people, but at ratios of working people to others that are themselves functions of the rate at which industrial productivity can be made to change. And is it not at least to be asked whether a properly dedicated Special Office (responsible to the Prime Minister) would find itself suggesting that by far the simplest population policy would consist of that direct device for limiting the rate of growth without prejudice to economic prosperity—finding some way of increasing the proportion of males in the newborn population?

The heresy that a more uniformly distributed population is a happier population needs also to be challenged, not of course because of the risk that the Special Office will actually be created but rather because this is already one of the determinants of social policy in Britain which hangs like a millstone around the country's neck. The distinction to be made is between a proper care for the integrity of communities threatened with the disappearance of their livelihoods because of industrial change, and the slavish attempt to make sure that there is never a radical transformation of the pattern on which the population is distributed. The last government was particularly keen on regional policies, but all British governments have had an unreasonable tendency to encourage people to keep on living in remote places such as the Scottish highlands when it might easily have been more suitable for those concerned as for the economy as a whole to have devised some way of helping people to move. In the years ahead, it may easily be much better for governments like the British government to seek ways of helping people to live more comfortably in very large cities than to find devices for dispersing them. The fashionable scorn for large urban communities is a dangerous trap into which, with an abandon that characterizes its latest report, the Select Committee on Science and Technology has lightly plunged.

New Ways with Defence Research

THE House of Lords last week (on May 12) took a first and probably a last look at the British government's plans for a new organization for the development and purchase—procurement as the saying goes—of new military equipment. Less than a year from now, on April 1, 1972, there will come into being a new kind of government organization called the Defence Procurement Organization. By this step, responsibility for new weapon systems and the research and development necessary to bring them forward will be transferred from the Department of Trade and Industry (itself a successor of the Ministry of Technology) to the Department of Defence, the real user of the weapons. Arrangements for defence research and

development may in this process be changed more radically than in any of the substantial reorganizations of defence procurement that have taken place in the years since the Second World War. There is also a chance—this is the apparently breathtaking claim which the Minister of Defence, Lord Carrington, made in last week's debate—that the British government will for a time at least now be prepared to refrain from tinkering with its organization for defence procurement.

The shape of the Defence Procurement Organization has been determined by the report of a working party under Mr D. G. Rayner published last month (Cmd 4641, HMSO, £0.35). The intention has been to create

an organization with an integral part in the Ministry of Defence (to which it will be responsible) and yet sufficiently strong and technically adventurous to be able to ensure that the design of new weapons is not excessively determined either by the immediate needs of the military services or by unrealistic technical ambitions. The difficulty of striking a balance between service interests and longer-term considerations is the reason why successive British governments have oscillated between arrangements which placed responsibility for procurement with the service departments and arrangements in which responsibility lay with an organization detached from the services and able to function as a kind of agent. The Ministry of Supply, which lasted until the early sixties, is recognized, in retrospect, to have become too unresponsive to military needs. Exactly similar, if more interesting, faults were apparent in the Ministry of Technology, which consistently muddled its responsibility for developing new weapon systems and its responsibility to safeguard the long-term health of British industry, including those parts of it involved in military developments. Mr Rayner's scheme is deliberately intended to be a judicious compromise, for the new organization will be firmly placed within the Ministry of Defence yet so well endowed with prestige and senior people—the top man will be called the Chief Executive and is obviously meant to be the sort of fellow who can talk back to all the service chiefs—that it will be free to make policy on its own account.

What does this imply for defence research and for the defence research establishments? Almost inevitably, the debate in the House of Lords last week was more concerned with issues such as the proper relationship between military and civil aircraft development (with civil aircraft being transferred to the Department of Trade and Industry) and with issues such as responsibility for the trouble about the Rolls-Royce engine for the Tristar aircraft than with the relationship between research and development in this important field (which costs the government more than £1,000 million a year). This is a great misfortune because the Rayner report makes radical proposals for the reorganization of defence research, not all of which are entirely to be welcomed. First of all, the Defence Procurement Organization is intended to consist of five operational divisions, one of which will be responsible (under a Controller) for research, development and the defence research establishments. On the face of things, this is a wise arrangement. There is everything to be gained from a close and equal partnership of research and development with the divisions of the new organization responsible for more immediate developments. It is also intended that the Atomic Weapons Research Establishment at Aldermaston should be transferred to the new organization. This is one of the points in the Rayner report that Lord Carrington and his associates could well have accepted last week, for it is evidently sensible that an essentially military laboratory should be a part of the Ministry of Defence. So far, it will be said, so good.

The dangers in the new proposals consist largely of the way in which potentially useful laboratories will now be more or less perpetually locked into an exclusively military organization. This is one of the penalties of the tidy arrangements which the Rayner report recommends. For all its faults, the old system under the Ministry of Technology did at least have the advantage of bringing

establishments such as the Royal Aircraft Establishment at Farnborough and the Royal Radar Establishment at Malvern, two of the most powerful organizations for research and development in Britain, into close contact with civil industry. By now, it is painfully apparent that both laboratories have much to contribute towards industrial development in fields quite separate from the military. In particular, there is the strongest possible case for asking that the Malvern laboratory should be made the centre for a determined assault on the long-term problems of the telecommunications industry. By comparison, its function within the apparatus of military procurement, though important, is comparatively less urgent. One of the points on which Lord Carrington should have been pressed last week in the House of Lords is precisely this danger that the Defence Procurement Organization will serve as an insulator between military and civil research and development.

100 Years Ago



THE SMALLER LECTURESHIPS AT THE LONDON MEDICAL SCHOOLS

II.—THE TRUE FUNCTIONS OF THE SMALLER SCHOOLS

IN a recent article* we pointed out the prodigious waste of time and energy that results from the existence of no less than eleven medical schools in the metropolis, with from thirteen to twenty-one lectureships attached to each, and called attention to a scheme by which it is proposed that an amalgamation should take place between several of them.

It is maintained by those who have proposed this scheme that by its means a reduced number of central institutions would be created in which the preliminary subjects of medical education, such as natural philosophy, mechanics, rudimentary chemistry, and botany, could be taught in a much more satisfactory manner than at present, since the increased value of the lectureships would enable the lecturer to devote more time to their preparation, and to supply much greater wealth of illustration, whilst the larger number of students in attendance would correspondingly stimulate his zeal. At the same time the smaller hospitals and schools might still fulfil a very important rôle as supplying the means for the practical or clinical study of disease—certain lectureships still remaining attached to them.

SIR JOHN HERSCHEL

FOR nearly one hundred and fifty years Europe has not seen a more accomplished philosopher than the great and good man whose mortal remains were last week consigned to their tomb in the national mausoleum, finding there a significant resting-place close to the grave of Newton. In sorrow and friendly reverence they were followed thither by nearly all that England values as the most eminent in the various domains of those many sciences which he, through a long life, had adorned and advanced.

From *Nature*, 4, 61 and 69, May 25, 1871.

OLD WORLD

POPULATION

Crying Halt in Britain

SETTING a thief to catch a thief, the House of Commons Select Committee on Science and Technology has now put forward a proposal that there should be a special office of government to formulate policy on the British population. The committee's recommendation is based on a public inquiry carried out early in 1970, in the months immediately before the change of government, and on a supplementary memorandum by the Government Actuary and the Registrar-General.

The committee says in its report (HMSO, £2.40) that in spite of all the errors of forward projection of the population, there is likely to be "a substantial and continuing increase in population" between now and the end of the century. In June 1970, the population of the United Kingdom was 55.7 million. In one period of twelve months, from the middle of 1967 to the middle of 1968, births (951,000) exceeded deaths by 292,000. The committee is clearly impressed by the fact that the average number of children is running at about 2.5 per family, rather more than the 2.1 per family at which the population would be stable. It says that in the past few years, there has been "a dramatic swing" to higher rates of marriage and to a tendency for people to marry younger. (Since 1931, the average age of marriage has fallen from 25.7 for women to 22.7 at present. The corresponding ages for men are 27.5 and 24.7.) The committee says that neither abortion nor the activities of the Family Planning Association would be likely to control the growth of population, but that reductions of mortality brought about by better medical treatment are unlikely in the future to stimulate the growth of population as they have done in the past—in the past thirty years, there has been an absolute reduction of more than three-fifths in infant mortality. The report also accepts the evidence put to it by Sir Solly Zuckerman that the net outward loss by migration, which seems to fluctuate between 20,000 and 65,000 a year, would have to be very much larger if it were to offset the natural increase. On this view, the extra thirteen million people likely to be added to the British population by natural increase between now and the end of the century would be offset only by a net outward migration of between 200,000 and 250,000 a year.

What does the committee suggest should be done? The committee takes no view of the question whether there

is an optimum population for a country such as Britain, although it quotes the views of several of those giving evidence at the public inquiry that an attempt at such a definition would be fruitless. In particular, it says that there is still room for growth in the production of food in Britain, that government departments were concerned at the need not to put excessive pressure on resources and, in particular, to work towards a more uniform spread of population, that there are unsolved problems in predicting the ways in which available jobs will satisfy future demands for work and that there is a need to consider the effects of pollution on the environment. With great delicacy, the committee refrains from coming down from the fence straddled by its report, but it does say that there is "public anxiety" about the consequences of a rapidly rising population and "a lack of urgency" in government departments and that the committee itself does not share "the complacent view" of many government witnesses. The committee goes on to say that it would like the government to set up a special office responsible to the Prime Minister for the coordinated study of population statistics, the study of the relationship between population and resources, to carry out appraisals of the plans of government departments from the point of view of population to advise the government on population policy and "to publicize the effects of population levels and their consequences, the rôle of family limitation and socially responsible parenthood".

SOVIET RESOURCES

Water in Wrong Places

by our Soviet Correspondent

WATER resources in the Soviet Union are once again the subject of considerable concern in the Russian press (see *Pravda*, 133; 1971). According to the latest estimates, the demand for water will rise in the next twenty years, to between two to two and a half times the present. By 1985, wide-scale irrigation and land-reclamation schemes will have more than doubled the amount of land under irrigation, to reach a figure of some 21 million hectares. Increase in the numbers of hydroelectric plants, the rapid expansion of industry, the growth of population and the greater demands of fresh water for fishery purposes are aggravating the ever increasing lack of fresh water.

One of the most serious problems is that the water reserves of the Union are not well distributed in relation to the population. Averaged over the

waters of the entire Union, there is a supply per head of 20,000 cubic metres per annum. For the southern republics, however, the figure is only 0.3–1.5 thousand m³, and for the Black Sea Basin only 1,900 m³ per annum. Furthermore, most of this water is discharged by the rivers during the spring floods, and present storage arrangements are inadequate to retain these precious waters until they can be used. The gradual drying up of the Caspian and Aral Seas as their rivers are depleted by irrigation and industry is a well known problem—even before the war, there were plans to remedy this by a system of canals which would divert the waters of the north-flowing rivers such as the Ob' and Enisei, so that they ultimately reached the Volga and thence the thirsty areas of the south. The survey for this vast scheme was begun in the middle sixties and completed just in time to be announced as a new achievement at the Party Congress in March this year. The problem of the Aral Sea and the water regime of Central Asia (where less than half the water required for irrigation is as yet available), however, is a continuing worry.

The directives of the current five-year plan contain a number of proposals for increasing the water supplies of the Union, including desalination schemes, the use of known underground waters and the prospecting for new subterranean supplies. According to Dr A. Voznesenskii, Director of the Institute of Water Problems of the Academy of Sciences of the USSR, the chief issue is, nevertheless, at present one of serious planning. "The water economy has entered into a new phase of development," he says, in which one must consider "the interaction of water problems over extensive zones . . . the necessity for complex use of water resources, and the necessity of considering problems of the water economy in very long-term views."

LISTER INSTITUTE

New Wing for High Flyer

THE Lister Institute marked eighty years of existence this week with the official opening of the Wolfson wing, five storeys and 25,000 square feet of additional laboratory space built on the side of its London headquarters in Chelsea Bridge Road. The new wing cost £370,000, most of which was provided in a gift from the Wolfson Foundation. Despite the intervention of a fire which delayed completion by about four months, research began in earnest in the new building early this year and now all but two of the floors are completely fitted out.

The new building should prove more comfortable for research than the old; in the end, space was so limited there that it was necessary to take over the director's vacant flat on the top floor and convert the kitchen, bedroom and bathroom into working areas. The department of virology, in the charge of Professor L. H. Collier, and the Medical Research Council Trachoma Unit are accommodated on the fourth and fifth floors. The animal house and ancillary services are located above, and on the ground floor there is a fine lecture theatre sponsored by the Worshipful Company of Grocers. In between, the first and second floors lie fallow at present. Eventually this space will accommodate a new department of experimental immunology, but a shortage of money means that these plans have had to be held in abeyance. Because the establishment of such a unit must cost in the region of £30,000, it seems likely that the governors of the institute, wise in the ways of research funding, will be on the look out for a suitably eminent immunologist around whom such a unit could be built.



Wolfson wing from Chelsea Bridge Road.

The Lister Institute is an unusual organization whose structure and functions can perhaps best be compared with those of the Pasteur Institute in Paris. Both run research departments of international repute largely on the profits made from pharmaceutical sales, and both have found it essential to manufacture their products at a site remote from their headquarters.

The Lister Institute seems to be the more competitive manufacturer. Some 72 per cent of the total income in 1970 was derived from pharmaceutical sales,

and in the manufacture of vaccines such as smallpox, typhoid, anti-tetanus, anti-scorpion and whooping cough, it has virtually a monopoly in Britain. This unhealthy situation was remedied to some extent a few years ago when Evans Pharmaceuticals, a subsidiary of Glaxo Ltd, undertook to manufacture some of the vaccines which had been hitherto the Lister's sole province, thus avoiding the risk of a breakdown on the Lister's manufacturing lines.

It is, of course, hard to assess the performance of an institute such as the Lister Institute, with commercial companies, but there is no doubt that Lister products are highly regarded in the pharmaceutical world, and that the little firm is in no danger of being crushed by the giant concerns. It remains to be seen if the institution's reputation for fine research will ensure that the vacant floors in its new wing are swiftly filled.

CONSERVATION

Sink or Swim

THE Research Unit on the Rehabilitation of Oiled Seabirds (RUROS) at Newcastle upon Tyne announced last week that its search for suitable cleaning agents is paying off. Two compounds in particular, a rather nebulously specified hydrocarbon "similar to paraffin" and Arklone P from ICI, leave the plumage both clean and watertight.

RUROS, which is based at the zoology department of the University of Newcastle upon Tyne, has been studying this problem since January 1970, when it was set up at the instigation of the Advisory Committee on Oil Pollution of the Sea on a five-year grant of £33,000 from six British oil companies.

The discovery of the new cleaning compounds is, however, in itself no final solution to rehabilitation problems. The birds must still be retained for a short recuperative period, during which they must be allowed unlimited access to bathing water, for if the cleaned plumage becomes contaminated with even small amounts of faecal matter its water repellent properties are lost. This procedure requires specially equipped accommodation which at present, except for the RSPCA's bird hospital at Mousehole in Cornwall, does not exist. At this stage it seems that if an oiling disaster occurred tomorrow, as well it might, the bird people would be caught almost as much on the hop as they were when the Torrey Canyon broke her back in March 1967.

This situation may be improved after Dr K. G. Gregory, RUROS's scientific

director, meets next week representatives of the Royal Society for the Protection of Birds to discuss the organization of field trials for the new materials. This is one step towards making a workable technique widely available but, sensitive to the criticisms of the value and feasibility of rehabilitation, Dr Gregory is anxious that his laboratory methods are proclaimed foolproof in the field before the details are released.

His sensitivity is well justified, for the subject is the centre of considerable controversy. Critics point out that the small fraction of the birds affected by an oceanic oil spill which reach the shore alive are always in an extremely debilitated condition. They are invariably suffering from oil poisoning, advanced malnutrition and often irreversible hypothermia. Many say that to attempt to resuscitate this minority is futile, and support for this comes from the ringing studies of the British Trust for Ornithology, and which indicate abnormally high mortality rates among rehabilitated seabirds after release. Advocates of rehabilitation, on the other hand, claim that some people will always attempt to clean oiled birds, and that anything that can make their efforts more successful is justifiable. Certainly, workable rehabilitation methods would be a useful backstop in those cases where entire local populations are involved, or where the future of rare species is threatened.

Even if this essentially curative approach can be condoned, however, there remain large and fruitful areas of research of a more preventive nature to be explored. Little is known about the behaviour and movements of bird populations at sea, for example: could birds in some way be "steered" away from polluted areas? From the work of Dr W. R. P. Bourne, perhaps the only man in Britain studying the normal ecology of birds at sea, may stem the possibility of charting and characterizing bird "risk" areas, a project in which the Nature Conservancy is also interested.

But if conservation is the chief concern, as it appears to be, would not an equally, if not more, profitable approach be to investigate ways of reducing the nesting mortality of seabird breeding groups? Replacement rates for auks, in particular, are notoriously low, and auks suffer more from oil pollution than any other group. Cleaning old nesting ledges and perhaps creating new ones may build populations to a point where the present decline, aggravated if not caused by oil pollution, would be halted. Reason might then hold its own with emotion in the arguments on rehabilitation.

NEW WORLD

St George versus a Gypsy Moth

by our Washington Correspondent

In the next few days, millions of be-whiskered gypsy moth caterpillars munching peacefully in the woodlands of Pennsylvania, New Jersey and New York will have their fate determined by a battle of words now in progress in Washington. In intellectual terms, the battle has been a walk-over for those who argue that gypsy moths may in all likelihood be less pernicious than the measures taken to exterminate them. But the Department of Agriculture will almost certainly go ahead regardless with its plans to spray the Union Carbide insecticide 'Sevin', or carbaryl, over some 400,000 acres of forest. The department, after all, like many bureaucracies, is kept in motion by force not of reason but of habit, and it has been eradicating the gypsy moth for so many years—more than a century—that the original *casus belli* has long been forgotten. In this 100-years war the USDA forces have fought long and valiantly, spraying thousands of acres of woodland with DDT before that became unfashionable and expending more than \$100 million in the process. The gypsy moth is as firmly established as ever, but who likes to admit defeat?

Certainly not the public relations people at USDA. Their handouts continue to make the gypsy moth sound like a visitation of Attila, even though other sections of the department have now abandoned this kind of propaganda. Gypsy moth caterpillars, says a recent USDA press release, "strip the leaves from forest, shade and fruit trees, as well as ornamental shrubs. By defoliating forests they increase fire and erosion hazards, adversely affect stream flow, reduce land and recreational values, and destroy wildlife habitats". To be sure, the caterpillars eat the leaves on the trees, but fire? erosion? These charges, which are repeated in a press release dated April 27, 1971, and issued by the Agricultural Research Service of the USDA, might be expected to figure prominently in the Environmental Impact Statement which, since January this year, federal agencies have had to file with the Council on Environmental Quality for any programme that may affect the surroundings.

Strangely enough, in the impact statement filed this month by the Forest Service of the USDA, there is no mention of fire or erosion hazards. Officials of the Forest Service, to which gypsy moth countermeasures have been handed over this year from the Agricultural Research Service, explain that

though the gypsy moth may increase fire and erosion hazards in some places, they felt "we couldn't generalize". The gypsy moth may no longer be a fire hazard, but the Environmental Impact Statement prepared by the Forest Service nevertheless makes it out to be a dangerous character.

Wood products are the most tangible of the forest resources the gypsy moth is alleged to devastate but even here the impact statement is unable to say how much timber will be lost or to give any cost-benefit figures for the programme. As a justification for the programme, the argument presented in the impact statement is thickly populated with weaknesses and impressions remarkable in an official document. Many of these weaknesses have been brought to light by the Environmental Defense Fund, an organization of 18,000 members that with varying degrees of success has challenged the USDA on its use of pesticides such as DDT and dieldrin, as well as carbaryl, the chemical now used against the gypsy moth.

Last month the fund submitted a list of some 35 comments and criticisms on the draft impact statement prepared by the Forest Service, only a few of which have been answered in the final form of the document. But unless the EDF were to succeed in taking out an injunction against the spraying programme, the Department of Agriculture will not be compelled to make its actions intellectually respectable.

Officials at the Council on Environmental Quality were apparently unhappy with the statement, but since it was only filed on May 12 and spraying has to begin within the next few days, the council decided not to ask for a delay. The Department of Agriculture, its blackmail successful, has assured the council that impact statements will never be filed so late again. In any case, the council has no legal powers to halt a programme and its only influence lies in persuasion.

The objections raised by the Environmental Defense Fund in its comments—acknowledged both in the Forest Service and the Council on Environmental Quality to be excellent—include questions on the population dynamics of the gypsy moth, the real nature of the damage suffered by forests, the effects of the carbaryl insecticide on wildlife and humans and the possible control methods alternative to that chosen by the USDA.

Many of these questions are devastat-

ing in their simplicity; for example, the EDF asks why the Forest Service does not wait until the gypsy moth population has reached a natural equilibrium, as it has done in Massachusetts. Ought the gypsy moth to be left alone so that this equilibrium might be reached more quickly? Is it possible that the spraying programme, by killing the natural enemies of the gypsy moth as well as the moths themselves, may simply postpone at great economic and environmental cost the natural evolution which must take place while gypsy moths find their natural niche? Why does the Forest Service not discuss the evidence that the survivors of a sprayed population are stimulated to lay a larger number of eggs and build up their numbers more rapidly than an unsprayed population? What of the evidence that by increasing the diversity of tree species in a forest, gypsy moth infestations make it more resistant to subsequent attacks? What of the other forest insects killed by the spraying? The report of the Mrak Commission on pesticides states that use of carbaryl around humans should be strictly limited—why is this recommendation not discussed? What percentage of the budget for the gypsy moth programme is spent on research? What effort is being put into the development of natural biological controls?

The impact statement prepared by the Forest Service answers few of these questions directly. The Forest Service agrees that the presence or absence of gypsy moths makes little long term difference to a forest—the purpose of the spraying is no more far reaching than to prevent the damage to this year's timber. The gypsy moth and all other species of wildlife will be back to their previous levels within three years of the spraying, the statement says. The Forest Service admits that carbaryl will kill aquatic insects in the area, but does not seem to believe the fish that feed on these insects will be affected. There have been "no strong correlations" between reduction of aquatic insect populations and fish kills, the statement observes, and "if there were such a correlation one would expect that fish killing would have been more common". In other words, presumably, since carbaryl does not always kill fish, therefore it never kills fish.

The impact statement also acknowledges that carbaryl kills many other terrestrial insects besides gypsy moths, such as true bugs, wasps, bees, ants,

other species of Lepidoptera, and gypsy moth predators such as lady beetles, parasitic wasps and bees and certain hemipterous and coleopterous predators. The insecticide also kills any honeybees their owners may not have shut in during the spraying, but "honeybee populations recover, frequently producing more honey than normal", the statement says. How long-lasting will these effects be? "To our knowledge," the anonymous authors of the impact statement remark, "no detailed studies have been conducted on the effects of carbaryl on terrestrial insects and fauna in the forest. Undoubtedly, many of these animals may be directly affected by the pesticide."

Although the Mrak commission recommendation is not mentioned in the impact statement, Forest Service officials claim that the recommendation does not mean what it appears to say. The commission includes carbaryl in a list of pesticides cited under the recommendation "Minimize human exposure to those pesticides considered to present a potential health hazard to man". The list is introduced, however, by the words "The evidence does not prove that these are injurious to man but does indicate a need to re-examine the registered uses of the materials and other relevant data in order to institute prudent steps to minimize human exposure to these chemicals". Forest Service

officials interpret this to mean that the chemicals in question have not yet been proven injurious to man and that until so proved may be used without restriction. Awkwardly for this interpretation, the list of chemicals includes in addition to carbaryl the herbicide 2,4,5-T, which at the time of writing was already known to present certain hazards and has since been restricted.

The Environmental Defense Fund has not been the only organization critical of the USDA impact statement. Robert J. Bielo, director of the Pennsylvania Fish Commission, considers that the programme will "cause modest to immediate and long range damage to the fishery resource of the area sprayed and in the water affected by the run-off due to the toxicity of carbaryl to valuable terrestrial and aquatic insects and plankton".

Bielo regards the programme as "designed to benefit a few resort areas, rather than to protect the forests. It is clear, he says in his comments on the draft impact statement, that "neither the using agencies nor the chemical industry has shown but minor interest in learning something of the overall environmental impact of this toxic chemical... The Environmental Impact Statement seems to be simply an exercise designed to justify the spraying rather than a straightforward presentation of known facts. In fact, the state-

ment fails really to make a case for spraying and provides more justification for not conducting the programme than it does anything else".

Why does the Department of Agriculture persist in pursuing a programme of which the objectives are ill-defined, the benefits clearly temporary, the biological consequences unknown because of inadequate research, and the intellectual foundations so shoddy as to destroy any pretensions to academic respectability the department might still hold?

One explanation is that the USDA still has not outgrown its frontier-spirit mission of loading up the spraying aircraft whenever an insect shows its head without taking further thought for the consequences. Another theory has it that most pest infestations are cyclical and the USDA has to magnify the damage caused by its insect enemies in order to keep its staff busy during the troughs between the infestation peaks. A third explanation, not incompatible with the previous two, has it simply that the USDA builds up insects like the gypsy moth and the fire ant into dragons in order that it may play the role of St George to a grateful and admiring public. The spectacle would not be so sad did the Department of Agriculture have any prospect of conquering its gypsy moth adversary with its present methods.

Jitters at Nader Raid

by our Washington Correspondent

THE consumer advocate Ralph Nader officially launched his investigation of the National Academy of Sciences last week by releasing the text of a letter sent on May 8 to Dr Philip Handler, president of the academy, and to Mr Clarence H. Linder, president of the associated National Academy of Engineering. In fact, the investigation began a month ago with a contretemps indicative of the academy's distinct nervousness at its impending ordeal.

Any organization would be alarmed at the opportunity of entering Nader's graveyard of institutional reputations, but the National Academy of Sciences in all probability has fewer skeletons in its committee-room closets than had previous victims of "Nader's raiders" such as the Food and Drug Administration, the Federal Trade Commission and the Federal Water Quality Administration. The academy won the distinction of being put on Nader's list of suspects not because of any scandalous neglect of the consumer's interests but by reason of a speech made by former Secretary of the Interior Stewart L. Udall suggesting

that Nader should sponsor a study of the academy.

Udall's suggestion was taken up by Philip M. Boffey, a journalist on the staff of *Science*, who proposed to Nader that he should carry out the study. Boffey is now directing the study on behalf of Nader's Center for

the Study of Responsive Law. Another probable reason for Nader's interest is the increasingly visible role of the academy in public affairs. The present controversies that dog almost every step taken by the Atomic Energy Commission have brought into prominence the academy's part in the



Nader and Handler—inquisitor and innocent.

setting of permissible radiation levels. The continuing tussle between the drug companies and the Food and Drug Administration has also pushed the academy into the limelight since it is on the basis of advice tendered by committees of the National Research Council, the operating arm of the academy, that the FDA has recently ordered scores of drugs off the market. A third and even more far-reaching responsibility which has been thrust upon the academy in recent months is the job of refereeing the automobile manufacturers' progress in reducing exhaust gas pollutants to the low levels demanded by the Clean Air Act. In Nader's eyes, a group that wields such apparently powerful influence over the affairs of the AEC, the FDA, Detroit, and all the consumer interests represented thereby is worthy of careful study.

Nader's letter to presidents Handler and Linder lays out the scope and purpose of the investigation in terms that reflect, though in more precise form, the chief criticisms made by Udall. Udall (see *Nature*, 229, 151; 1971) claimed that the academy "by confining itself to a clientele almost exclusively made of government agencies, and by permitting its clients to phrase the questions that it will study, has all too often become a mere adjunct of established institutions". Nader explains his reason for launching the study as that "the academy, through its role as an official adviser to the government, is in a position to exert great influence over public affairs, yet its activities go largely unmonitored. . . . You have great potential power yet little or no responsibility to answer to the public for how that power is used".

Knowing the exact nature of the charges preferred has done something to soothe the academy's feelings. "We are willing to cooperate with the Nader study to any reasonable extent," a staff member of the academy said last week. But the academy feels unable to follow the example of government agencies which have opened up their files and internal documents to the candid appraisal of Nader's study teams. As a private organization, its spokesmen argue, the academy has been entrusted with proprietary information by manufacturers and with private judgments on colleagues by individuals; for this reason the academy's internal correspondence and the minutes of its committees must remain privileged material.

Boffey, who is undertaking the study singlehanded except for the assistance of two students during the summer—Nader raids are usually mounted by posses of a dozen or more people—says that the academy is being "reluctantly and apprehensively cooperative". The

academy is making people available for interviews, but has sent all its staff members a memorandum laying down the rules for the interviews, one of which is a warning that any document composed within the last 50 years may be in the privileged category. Boffey has not yet met Handler; he and Nader arranged to pay a courtesy call on Handler a month ago to explain the nature of the study, but at the last minute Nader was unable to keep the appointment and Handler, learning that Boffey had arrived at his office alone, refused to see him.

Another source of unease between the academy and the investigator is the former's worries that it may not get fair treatment. The Nader study is to be published, and the academy's fear is that to produce a saleable book, Boffey may be drawn to emphasize the

negative aspects of what he finds. "It is as much in Nader's interest to produce scandal as it is in a contemporary movie to show bosom," a staff member of the academy told *Science News* last month. "That just shows when they last saw an X-rated movie at the academy," Boffey retorts, adding that he sought and received assurances from Nader that the study does not have to be negative. Boffey says he will write about any scandals he may find, but the study is intended to be objective, and it is "not inconceivable to me one could find it's a pretty good institution". The academy seems to have come round to this view; "Boffey is interested in the things we are proud of as well as those that didn't turn out too well. I think he will be fair to us in his lights and I am satisfied with that," a staff member said last week.

Nader's En Garde to Handler

Dear Dr Handler,

I am taking this opportunity to inform you that the Center for Study of Responsive Law is sponsoring a study of the National Academy of Sciences—National Academy of Engineering—National Research Council. The study will focus primarily on the rôle of these institutions in helping to shape public policy on issues which involve science and technology—issues such as pollution, pesticides, nutrition, housing, drugs, the SST, airport expansion, transportation, and military affairs, among others.

Our reason for launching this study is that the Academy, through its rôle as an official adviser to the government, is in a position to exert great influence over public affairs, yet its activities go largely unmonitored. Your key committees deliberate in private, the minutes of your meetings are withheld from the press and the public, and the nature of your advice to government agencies and other clients is often considered privileged information. You have great potential power yet little or no responsibility to answer to the public for how that power is used.

Our study will attempt to measure just what contribution the Academy has made on public issues. We will examine such questions as what problems the Academy has tackled, what advice it has offered, what impact that advice has had, and what factors resulted in the advice being accepted or rejected. The study will also attempt to make some value judgments as to how well the Academy has performed as an adviser on public issues. We will also examine such questions as how a decision is made to undertake a project, how a committee is selected to carry out the project, and how a consensus is reached on a committee. We will also try to determine to what extent the Academy serves the broad public interest and to what extent it serves the narrower interests of its members, or of the scientific community, or of the government agencies which pay for its advice, or of other identifiable interest groups, such as industry. Though the study will focus on the official advisory rôle of the Academy, it will undoubtedly delve into other aspects of Academy operations so as to give a rounded picture of the institution.

The study will be directed by Philip M. Boffey, a former staff writer for *Science* magazine, the official journal of the American Association for the Advancement of Science. Mr Boffey will be assisted by several qualified students.

I trust we can count on your cooperation.

Sincerely yours,

Ralph Nader.

NEWS AND VIEWS

The Exploration of the Outer Planets

THE discovery by Kemp and his colleagues (see page 169) of circular polarization on Jupiter is a reminder not merely of the problems of the outer planets which have been crying out for a solution for several years but also that much will be done in the years ahead to define problems as yet unrecognized. Classical methods of observation will no doubt contribute in important ways, but it is not too soon to look forward to the spate of information that will presumably be produced by the Grand Tour of the outer planets, a reconnaissance mission which is currently being planned by NASA. The gravity field of Jupiter would be used to deflect each spacecraft toward two more outer planets. Spacecraft launched in 1976 and 1977 would go past Jupiter to Saturn and Pluto. Spacecraft launched in 1979 would encounter Uranus and Neptune, after leaving Jupiter. Because the last encounter would take place at extremely great distances (30 AU), the typical mission would last about ten years.

The mission will investigate fundamental questions regarding the origin and evolution of the solar system. The little that is known about the outer planets shows them to be very unlike the Earth or its nearest companions, Venus and Mars. Although very large, the outer planets are less dense than our own Moon, and it thus seems likely that they are composed principally of hydrogen, perhaps in a fluid or even a solid state. Their composition is believed to approximate closely that of the primordial nebulae from which the Sun and planets presumably accumulated. Information regarding their composition could thus throw some light on the abundance of the elements in the original solar nebula.

The large size and mass of the outer planets have retarded the loss of their atmospheres, unlike the inner planets the atmospheres of which were too hot and gravity fields too weak to retain their primordial atmospheres. Furthermore, the Earth's atmosphere has been drastically modified by life processes. Thus, the atmospheres of the terrestrial planets are almost devoid of hydrogen and helium, which are expected to be abundant in the atmospheres of the outer planets. One goal of the mission would undoubtedly be to measure the ratio of hydrogen and helium at all the giant planets. This could be accomplished by measurements made in diverse parts of the electromagnetic spectrum such as the amount of solar ultraviolet scattered by the upper atmosphere, the infrared radiance from the lower atmosphere, and the dispersion of the radio waves using the radio signals of the spacecraft.

The outer planets may retain other traces of the early history of the solar system. The Earth's atmosphere must originally have been similar in composition to the present atmospheres of Jupiter and Saturn. Complex organic molecules may be formed in such atmospheres and on Earth may have been precursors to life. Such molecules have been invoked to explain the unusual colouring of the clouds that cover Jupiter and Saturn. Organic molecules may be created in hydrogen-rich atmospheres, perhaps by the passage of a lightning discharge. Simple detectors on spacecraft passing near Jupiter and Saturn could investigate the possible occurrence of lightning, and more

complex infrared instruments could investigate the origin of the colours often seen in the clouds.

The measurement of infrared emissions from the atmosphere has other important implications for outer planet research. Earth-based measurements indicate that both Jupiter and Saturn have an internal source of energy and they radiate significantly more energy than they absorb from the Sun. It is possible that both planets are contracting slowly and gravitational energy is being converted into heat, but this question cannot be answered from Earth because of the inability of an Earth-based observer to view the dark side of the outer planets. Careful measurements by spacecraft of the relative amounts of energy reflected from the planets in the visible and radiated into the infrared may settle this question.

The outer planets apparently differ from the terrestrial planets other than Earth in yet another way. Earth is the only inner planet having a significant magnetic field and trapped radiation. Radio astronomical observations at long wavelengths indicate that Jupiter is one of the brightest radio sources in the sky. Studies of these intense planetary emissions, and those at shorter decimetre wavelengths, imply that Jupiter has a planetary magnetic field at least ten times stronger at its surface than the Earth's field. The field is so intense and the planet is so large that the magnetic field of Jupiter occupies an enormous volume relative to that in which the Earth's radiation belts are confined. The Jovian radio noise is undoubtedly radiated by energetic electrons trapped in this voluminous magnetic sphere of influence.

Direct measurements of trapped radiation near other planets are probably required in order to understand fully the Earth's radiation belts. The origin of the particles and their subsequent acceleration to high energies is still a matter of keen scientific interest. The trapped energetic particles seem to be extracted by some mechanism from the solar wind and the ionized gas escapes continuously from the Sun's corona, which fills the inner solar system. One of the scientific studies carried out at the outer planets would undoubtedly be the nature of their interaction with the solar wind. Furthermore, the properties of the Jovian trapped particles could be measured, and trapped radiation at the other giant planets could be sought by particle detectors. Particle trapping in large scale magnetic fields seems to be commonplace in the universe, as witness their role in recent attempts to explain pulsars.

A favourable conjunction of the outer planets making multiplanet missions of this kind possible occurs roughly every 175 years. Will the United States celebrate its 200th anniversary in 1976 by embarking on this ambitious undertaking? There are obviously many scientists in Britain and Europe who are willing to join the celebration by becoming involved in the mission. Although the interval between the first and last encounter would be eleven years, the set of four launches presently being studied would imply a rendezvous with at least one of the outer planets in seven of those years. Such a prospect would maintain a high level of interest in outer planet research throughout the world.

ENZYMES

Numinous Nuclease

from our Molecular Biology Correspondent

A **SIZABLE** textbook could now be written about bovine pancreatic ribonuclease: it would encapsulate the most striking achievements of modern enzymology, protein chemistry, and X-ray crystallography. Last year the total synthesis of active ribonuclease was reported simultaneously by two laboratories. The full details have now been described by Gutte and Merrifield (*J. Biol. Chem.*, **246**, 1922; 1971), in an article the length of which reflects the magnitude of the undertaking. It is mandatory reading for anybody interested in the state of the craft of solid-phase peptide synthesis — the procedure developed by Merrifield, and representing one of the most important technical advances in biochemistry of recent years.

The scope of the method is limited by the efficiency of each peptide addition step, and to a lesser extent the ease of cleavage of the finished product from the column support, and of the protecting groups from side chains. By dint of a variety of minor modifications, the loss at each addition cycle was brought down to a level of 1.4 per cent, which corresponded to a yield of the final product of 124 residues of 17 per cent. Cleavage from the resin and deprotection were accomplished in a single step with a maximal efficiency of 63 per cent. Oxidative formation of the correct disulphide pairings, followed by ion-exchange chromatography, gave a highly active product; this was then further fractionated by treatment with trypsin, to which native ribonuclease is highly resistant, so as to remove any incorrect, and by implication improperly folded, contaminants, and this was followed by a fractional ammonium sulphate precipitation. The resulting synthetic enzyme had an activity of 78 per cent, compared with the native crystalline preparations, and by a series of chemical and enzymological criteria the two were indistinguishable.

Some interesting applications of such syntheses are not slow to suggest themselves, and one is to prepare selectively aberrant chains, in which residues are changed, omitted or added at will. One such exercise is described by Gutte and Merrifield. Ribonuclease S is a form containing a break in the polypeptide chain between residues 20 and 21. Since the separated S-peptide (1-20) can be restored to its position in the protein with regain of activity, it has been possible to evaluate the contribution of particular side chains in the peptide towards correct folding and activity, by studying the interaction of S-protein with modified S-peptides. Gutte and

Merrifield have now instead modified the S-protein by terminating the synthesis after ninety-nine residues, thereby eliminating five residues, including one buried tyrosine. S-peptide not only restores the activity by combining with this truncated S-protein chain, but its presence also permits it to refold correctly from the reduced state. The missing pentapeptide has at least two residues that seem to be involved in a system of hydrogen bonds to other parts of the chain. Evidently a partial disorganization in this region of the enzyme has no effect on the integrity of the active site.

A method that has been widely used to observe interactions at the active site is proton magnetic resonance. A difficulty associated with this technique in general is to disentangle and assign resonances arising from particular side chains. A new approach, which serves to simplify the picture, and to visualize events only as they affect a particular side chain, has been attempted by Huestis and Raftery (*Biochemistry*, **10**, 1181; 1971). They have studied magnetic resonance of the fluorine nucleus, this being introduced into the protein by trifluoroacetylation of lysines. Both the lysines of S-peptide can be modified in this way, without detriment to its ability to recombine with S-protein. Three resonances, two from the ϵ -amino substituents and one from the α -amino, can be assigned, and the first two shift and one splits on recombination of the peptide with S-protein. Introduction of inhibitors, including the phosphate ion, causes the perturbation only of a signal from lys-7. From the structure of the enzyme, and the pH profile of the chemical shift, Huestis and Raftery deduce that the inhibitors effect a small displacement in the his-12 imidazole, which probably depends on the protonation state of the opposed active site

imidazole of his-119. This type of approach can also be used (perhaps as rather a hard way) to study binding equilibria. Lee and Chan (*Biochem. Biophys. Res. Commun.*, **43**, 142; 1971) have used ^{31}P nuclear magnetic resonance to observe the binding of UMP to ribonuclease. The broadening of the resonance from the inhibitor is used to obtain a binding profile and an association constant. The broadening is related to the rate of exchange of ligand at the binding site, and can be used to obtain a dissociation rate constant, which agrees moderately well with directly measured values.

INTERSTELLAR MOLECULES

Sulphur—at Last

ONE of the more elusive atoms in interstellar space has now been tracked down. In a recent *International Astronomical Union Circular* (No. 2322), P. M. Solomon, of Columbia University, reports observations of three new interstellar molecules, carried out in collaboration with K. Jefferts, A. Penzias and R. Wilson, of the Bell Telephone Laboratories.

Using the National Radio Astronomy Observatory's 36 foot (11 m) antenna at Kitt Peak, this team has found methyl cyanide (CH_3CN), carbonyl sulphide (OCS) and carbon monosulphide (CS) in some of the well studied interstellar clouds. The Sagittarius complex has again proved a fruitful source, with transitions corresponding to $J=6-5$ ($K=0$ and 1) for CH_3CN appearing in the spectra of both Sgr A and Sgr B2 at 110.383 GHz and 110.381 GHz.

But it is the long awaited discovery of sulphur which is the most interesting feature of this report. In the spectrum of Sgr B2 (but not in Sgr A) there is a feature attributed to the OCS

Thermal History of Lunar Rocks

AFTER examining a sample of lunar basalt, S. S. Hafner and his colleagues at the University of Chicago report in next Monday's *Nature Physical Science* that annealing of rock from the surface of the Moon took place at about 600° C. This is shown by sub-microscopic unmixing and ordering of Mg and Fe among the cation sites in the mineral which was present as large, probably equilibrium, crystals suspended in lava when it was erupted from the lunar surface at about 1,150° C. This effect is not observed in terrestrial lavas with simple cooling histories. Slow cooling in thick flows might lead to the observed effects, but more often leads to microscopic unmixing and physical separation of the two components from high temperature crystalline phases in

the original homogenous state. Similar effects are known in ancient terrestrial igneous rock, particularly in dykes cutting gneisses in continental shield regions, where the rock has been rapidly cooled and then reheated or held for long times at moderate temperatures.

At a recent International Astronomical Union symposium at Newcastle upon Tyne (see *Nature*, **230**, 491; 1971), there was discussion of evidence showing the real age of lunar lavas to be 4.5×10^9 years, implying that the $3.3\text{--}3.7 \times 10^9$ year ages, widely reported for the formation of the groundmass minerals in the rocks, must refer to a subsequent thermal event for which Hafner *et al.*'s results might provide independent evidence.

$J=9-8$ transition at 109.463 GHz, and CS has been detected by its radiation at 146.969 GHz (corresponding to the $J=3-2$ transition) in no less than four sources—Ori A, W51, DR 21 and IRC+10216—but not, as yet, in either Sgr A or Sgr B2.

The Doppler shifts of the spectral features of all these new molecules are in the range already determined by measurements of the radiation from other molecules in these clouds. This report brings the total number of molecules identified in interstellar space to eighteen, the majority found within the last few months, and there seems every reason to believe that the number of identifications made will continue to increase rapidly.

But in the current issue of the *Monthly Notices of the Royal Astronomical Society* it is reported that a search for a further interstellar molecule containing sulphur, thioformaldehyde (H_2CS), has proved negative (152, 7p; 1971). R. D. Davies, R. S. Booth and A. Pedlar used the 250 foot radio telescope at Jodrell Bank on May 6-7 last year to search for a 1046.48 MHz line, corresponding to the $1_{10}-1_{11}$ transition. But there was no sign of the line, in either emission or absorption, in the directions of Sgr A, Sgr B2, M17 or Cas A. A similar negative result from a search for the same transition of H_2CS has already been reported by N. J. Evans, C. H. Townes, H. F. Weaver, and D. R. W. Williams of the University of California, using the 85 foot antenna at Hat Creek (*Science*, 169, 680; 1970), but the Jodrell Bank limits are claimed to be lower.

The non-detection of H_2CS is surprising by analogy with the abundance of interstellar formaldehyde (H_2CO). The corresponding $1_{10}-1_{11}$ transition of H_2CO has been detected in something like fifty sources, but even accepting the cosmic abundance ratio for oxygen to sulphur of fifty to one it is odd that the transition in H_2CS has not been observed. There is no reason, of course, for supposing that the abundance ratio of H_2CO to H_2CS is the same as the ratio of oxygen to sulphur, and astronomers—and chemists—will be devising ways in which the discrepancy might occur. Much will depend on the nature of the ultraviolet radiation from stars that is incident on the clouds where H_2CO occurs, for the radiation might selectively dissociate H_2CS before it has chance to form. Obviously there is a great deal to be learned about the conditions in regions where comparatively complex molecules occur, but fortunately there is also plenty of information waiting to be deciphered in the signals from the molecules that have already been detected.

TUMOUR VIRUSES

Obstacles to Infection

from our Cell Biology Correspondent

MOUSE mammary tumour virus has the distinction of being the only RNA tumour virus which induces a carcinoma and at least some strains of the virus are transmitted vertically. As such it provides a model system for studying virus induced carcinomas, and carcinomas are, of course, the most prevalent forms of cancer of man. The only snag, and it is a great one, to mouse mammary tumour virus is that, to date, nobody has succeeded in infecting cells *in vitro* with it. To be sure, cell lines which produce the virus have been established from mammary carcinoma tissue and they continue to produce virus which will infect mice but not the stable cell lines currently available. It seems that this virus is fastidious about the type and state of differentiation of its host cells and their hormonal environment. Clearly until an *in vitro* cell system which can be infected and transformed is found, progress towards unravelling the biology of this virus will be slow. In numerous laboratories the search is now on for

such a cultivated susceptible cell and the report of Lasfargues, Kramarsky and Moore (*Proc. Soc. Exp. Biol. Med.*, 136, 777; 1971) should excite considerable interest.

Lasfargues *et al.* have studied the consequence of fusing epithelioid cells of the line CCL-51, which was isolated in 1962 from a spontaneous mouse mammary carcinoma and has produced mammary tumour virus ever since, with two other mammary cell lines, the murine line MG4 and the rat line RMG. These two lines contain mixed populations of fibroblastic and epithelioid cells and do not produce any virus. The origin of the cells in multinucleate heterokaryons obtained by fusing CCL-51 to either MG4 or RMG cells was determined by the morphology of the nuclei, and production of mouse mammary tumour virus was assayed by an immunofluorescence method using specific anti-mouse mammary tumour virus antisera.

Lasfargues and his colleagues found that although some 10 to 25 per cent of the cells in a culture of CCL-51 produced virus and could be stained with the specific antiserum, after fusion with MG4 cells some 63 to 84 per cent of the cells, including heterokaryons,

Seeking C Type Particles

ONE of the skeletons tumour virologists prefer to keep closely locked in their cupboards is the simple fact that almost all tumour inducing viruses cause sarcomas or leukaemias—cancers of mesodermal tissues. But in human populations only about 10 per cent of cancers are sarcomas; the other 90 per cent are carcinomas—cancers of tissues of epithelial, not mesodermal, origin. Does that mean that the virologists are barking up the wrong tree? Obviously part of the answer to this disturbing question depends on proving that carcinomatous tissues either contain or lack viruses closely related to the familiar sarcoma and leukaemia viruses. That is what Mitchell and his colleagues have been doing as they report next week in *Nature New Biology*.

All the known mammalian sarcoma and leukaemia viruses have a similar structure, the so-called C type morphology. Each roughly spherical particle consists of a central core or nucleoid which contains the RNA genome associated with protein in a complex of ill-defined structure. This nucleoid is enclosed in a unit membrane envelope acquired as the particle buds from the surface of infected cells. All these viruses can be characterized in addition by their antigenic composition; the viruses from any one species have diagnostic group antigens and all the mammalian sarcoma and leukaemia viruses also share a common antigen.

Mitchell and his colleagues searched for viruses with these characteristics in two lines of hormone-secreting cells, one derived from a carcinoma of the pituitary, the other from an adrenal carcinoma. They found typical C type particles free in the culture fluids of the cells and also, in the electron microscope, they saw particles budding from the cell's surfaces. Furthermore, immunochemical tests as well as a biological assay for mouse leukaemia viruses indicated that these particles were closely related, if not identical, to mouse leukaemia virus.

Interpreting these results is complicated by the fact that the cell lines have been in culture for many years. As Mitchell *et al.* are well aware, it is impossible to implicate the virus particles in the aetiology of the original adrenal and pituitary tumours because of the long lapse between establishing the lines and detecting the particles. They do not believe, however, that the particles are adventitious or simply passengers. Rather they suggest that their findings support the oncogene hypothesis of Huebner and Todaro. This says that cells have in their genomes genes which can transform a cell to cancer, carcinoma or sarcoma, and can also in some circumstances develop into extracellular virus particles with a C type morphology. Mitchell *et al.* suggest this is what has happened in their two cell lines.

stained. Because the number of cells staining exceeded the number of heterokaryons and CCL-51 cells in the cultures, they conclude that some unfused MG4 cells were infected and produced progeny mammary tumour virus. On the other hand, when the CCL-51 cells were fused with rat RMG cells, only some 15 to 28 per cent of the heterokaryons and unfused cells stained. This suggests that the rat RMG cells neither induced the increased production of the virus by CCL-51 producer cells nor were productively infected themselves. In other words, the mouse mammary tumour virus seems to be species specific.

In the same issue of the *Proceedings* (*ibid.*, 742), Scher, Takemoto and Todaro report an interesting attempt to detect supertransformation of 3T3 mouse cells by SV40. It is well known that hamster cells transformed by SV40 can be transformed subsequently by polyoma virus. Scher and his colleagues have therefore asked whether a cell transformed by SV40 can be superinfected and supertransformed by more SV40. They selected lines of SV40 transformed 3T3 cells, which in spite of being transformed grow to comparatively low densities, and infected them with various amounts of SV40 virus to see if the superinfection changed the growth properties of the cells, causing them to grow to greater densities. But such supertransformation did not occur. They then selected lines of SV40 transformed 3T3 cells from which the transforming genome cannot be rescued by fusion with permissive African green monkey cells. These cells were then superinfected with plaque mutant strains of SV40, cultivated for a month or more, and were then fused with monkey cells. If the superinfecting plaque mutant genomes had stably associated with the cell genomes and persisted, they should have been rescuable, but none were. It seems therefore that once a cell has been transformed by SV40, further SV40 virus cannot infect and form a stable association with the genome of the transformed cell.

NUCLEAR PHYSICS

Polarized Neutron Source

from a Correspondent

A MUCH needed advance in the technique of producing polarized neutrons has been made simultaneously at the University of Birmingham and Los Alamos. The basis of the development, which will allow the production of neutrons of high polarization over a wide range of energy, is the observation that when polarized deuterons are accelerated on to a deuterium gas target the neutrons produced at zero degrees with respect to the incident

beam direction inherit 90 per cent of the vector polarization of the incident deuterons.

It has been known for a long time, of course, that polarized neutrons can be obtained from the $D(d,n)He^3$ reaction even when unpolarized deuterons are used as projectiles. The essence of the present development is that the polarization of the neutrons is independent of energy over a substantial energy range; the Birmingham group reports that the polarization of the neutron is constant over the deuteron energy range of 4 to 16 million electron volts (MeV). A further advantage of the method is that the energy resolution of the outgoing neutron is dependent only on the energy spread of the incident deuteron and the thickness of the gas target. With the recent developments in charged particle polarized beam production it thus seems likely that a beam of 10^8 neutrons per second of 90 per cent polarization will be available in the near future.

What will the experimental physicist do with these neutrons? The quality of data obtained with neutrons as pro-

jectiles has always been inferior to the corresponding data obtained with charged particles, and data obtained with polarized neutrons are almost nonexistent. The availability of a source of polarized neutrons thus opens out a completely new field of research. As well as allowing experiments in all branches of nuclear spectroscopy, such a beam of polarized neutrons coupled with a polarized target will enable a detailed study to be made as a function of neutron energy, of the neutron-nucleus spin-spin interaction. The ease with which the neutron spin direction can be altered by merely changing the conditions of the deuteron source will be a great boon.

Physicists will no doubt be wondering about where such a facility will become available. At Los Alamos a polarized charged particle ion source is already in operation on the tandem Van de Graaff accelerator. Unfortunately, the University of Birmingham does not have a suitable accelerator to exploit the new technique. It would thus seem a good idea that one of the tandem Van de Graaff laboratories in Britain should adopt the method.

Mapping the Surface of the Cancer Cell

THE chemistry of the surfaces of cells—particularly cancer cells and cells transformed *in vitro* by tumour viruses—seems destined to become one of the great bandwagons of biology in the next few years. Biochemists and tumour virologists are clearly going to have plenty to chew on and not a few may be expected to retire with indigestion, for it is becoming increasingly clear that transformation to malignancy causes radical but subtle changes in the chemistry and architecture of pre-existing membrane components as well as inducing the synthesis of quite new antigenic determinants. In the past months the changes in the surface architecture which can be monitored by measuring a cell's response to plant agglutinins have excited great interest and the experiments Burger reports next week in *Nature New Biology* are certain to keep the pot boiling.

He provides convincing evidence that Forssman antigen, a glycolipid with a terminal N-acetylglucosamine residue, exists in a cryptic state in untransformed hamster cells and is exposed as a result of transformation by oncogenic viruses. The fact that transformed cells give a positive reaction in tests for Forssman antigen, whereas comparable untransformed cells do not, is not news. Until now, however, it had always been assumed that transformation somehow switches on the synthesis of this determinant. But that is clearly not the

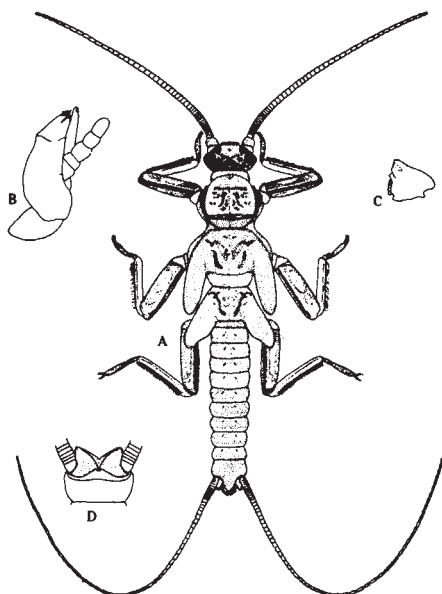
case, for, as Burger shows, in the same way that he showed that agglutinin sites exist but are cryptic in untransformed cells, exposure of untransformed hamster cells to dilute solutions of proteases reveals the same amount of Forssman antigen as does transformation. The antigen must be present in the surfaces of untransformed cells but somehow masked.

Even more intriguing perhaps is that Burger has managed to make a start towards mapping the proximity of this antigen to the receptor sites of various plant agglutinins. He finds, for example, that when Forssman positive cells are exposed to the agglutinin extracted from lentils, *Lens culinaris*, and then assayed for Forssman antigen the amount of antigenicity detectable is markedly reduced. Pre-exposure to concanavalin A, wheat germ agglutinin or soybean agglutinin, by contrast, has no such blocking effect. The obvious conclusion is that the receptor of lentil agglutinin is close to the Forssman antigen whereas the dispositions of the antigen and the three other agglutinin receptors are unrelated. It does not take much imagination to see where this approach to the architecture of the surfaces of transformed and untransformed cells is leading. It may not be long before there are cell surface maps which, crude though they will undoubtedly be, will nonetheless mark the relative location of a whole series of antigens and other surface markers.

PLECOPTERA

New Australian Species

LIKE mayflies, stoneflies (order Plecoptera) pass through an aquatic nymphal stage commonly found in streams and rivers, particularly those with stony or gravelly bottoms. Of the nine or so families in the order, one, the Gripopterygidae, is found only in Australia, New Zealand and South America (though there is also



one doubtful record from Fiji). Twenty new Australian species have now been added to the family list by I. D. McLellan who, in a revision published in the *Australian Journal of Zoology* (Suppl. No. 2; 1971), describes the new types which include the new genus and species *Neboissoperla alpina*. The larva of this species, shown here, was found in a small stream on Mt Wellington, Victoria.

COMPACT GALAXIES

Gamma Rays from 3C 120

from a Correspondent

AFTER the discovery of the first γ -ray star by Frye *et al.* (*Nature*, **223**, 1320; 1969), it seemed inevitable that somebody would eventually find γ -rays from discrete sources located outside our own galaxy. Surprisingly, the first extragalactic γ -ray emitter has been tracked down by a group of engineering physicists in the Soviet Union, who have just announced that the compact galaxy 3C 120 is a powerful source of 100 MeV photons. With this spectacular advance, the mystery of variable radio galaxies (see *Nature Physical Science*, **231**, 22; 1971) deepens yet further.

Unlike X-ray astronomy, γ -ray astrophysics is taking several years to build

up momentum, and there have been few big discoveries. Now that increasing emphasis is attached to satellite-borne telescopes permanently aloft, the pace will almost certainly quicken. The γ -ray observations of 3C 120 were in fact conducted with special equipment flown on the Cosmos 251 and Cosmos 264 satellites. These two probes are designed to make a slow rolling scan of a narrow band of sky on each orbit of the Earth. When S. Volobuev, A. Gal'per, V. Ugryumov, B. Luchlov, and Yu. Ozerov (Moscow Engineering Physics Institute) came to analyse the results, they found that some of the scans gave an enhanced count in a certain zone of the sky (*JETP Lett.*, **13**, 28; 1971), and they adduce this count of celestial γ -rays in excess of the background as evidence for a discrete source. The count rate is about one photon at 100 MeV or higher every 90 s, and this is ten times the rate for Sgr γ -1, the discrete galactic source.

Unfortunately, the precision of the positional information is rather poor, and they can only locate the source in a box 20° by 5° ; this is the stage that radio astronomy reached in the late

1940s. All is not lost, however, because the violently variable galaxy 3C 120 lies in the very centre of the error box. Furthermore, the γ -rays were detected at a time when the radio flux density measurements indicated the presence of a vigorous outburst of energetic activity. Finally, the measured γ -ray flux of 2×10^{47} ergs per second agrees beautifully with a prediction for 3C 120 made by the distinguished Soviet theoretician Iosif Shklovskii.

The theorists should now have plenty of fun because 3C 120 has been extensively observed in the radio and visible regions. A fundamental question is the nature of the γ -ray emission mechanism. One possible process is the inverse Compton effect, in which high-energy electrons scatter their own synchrotron radiation into the γ -ray spectrum. Here the snag is that the energy losses in the electron spectrum proceed at a catastrophic rate, so that the supply of relativistic electrons is rapidly exhausted. On the other hand, it is possible that relativistic energy is reinjected during the annual outbursts of 3C 120 and thus keeps the fires burning.

Insulin Activates Pyruvic Dehydrogenase

THE report by R. M. Denton and associates in next Wednesday's *Nature New Biology* that insulin may directly stimulate the activity of pyruvic dehydrogenase in adipose tissue is yet a further piece of evidence that not all effects of insulin are explicable by some primary stimulus to glucose metabolism. The Bristol group incubated rat adipose tissue with low concentrations of insulin and found a consistent stimulation of pyruvic dehydrogenase activity with glucose or fructose in the incubation medium. Stimulation also took place in the complete absence of any substrate. Such results may well explain that the known very high rate of glucose transport into adipose tissue (which would be expected to be stimulated by insulin) is not by itself great enough to account for the increased synthesis of fat. Control of fatty acid synthesis from glucose by insulin would then be exercised both at the cell membrane level as well as at a later key point in glucose metabolism, involving the fate of pyruvate.

Though it is fifty years since insulin was discovered, there is still widespread disagreement and argument about its mechanism of action. Because it was the simplest parameter of insulin action to be measured, its lowering effect on blood sugar levels was for a long time thought to be fundamental to all its other effects. In the past few years, however, it has been shown that several of the other metabolic activities of insulin apparently do not depend on glucose

metabolism for their expression. Such effects include the release of fatty acids from adipose tissue, as well as the important stimulatory effect of insulin on protein synthesis.

How then can these diverse modes of insulin action be explained on any unitary hypothesis? Some of the effects of insulin certainly seem to involve a depression of cyclic AMP levels following exposure of these tissues to insulin, as seen in liver tissue where depressed levels of cyclic AMP seem to depress glycogen phosphorylase and stimulate glycogen synthetase very soon after its administration. Such enzymes exist in two forms, a phosphorylated and a dephosphorylated form, and phosphorylation involves the activity of a cyclic AMP activated kinase. It is of especial interest therefore that two forms of pyruvate dehydrogenase have now been reported to exist in some tissues by Lin and his co-workers (*Proc. US Nat. Acad. Sci.*, **62**, 234; 1969). Once again one form of the enzyme is phosphorylated and the other nonphosphorylated.

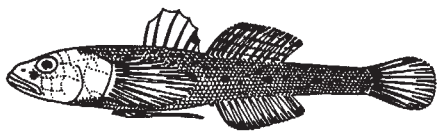
Though it might be very tempting therefore to ascribe the effects of insulin on this enzyme to a cyclic AMP mediated phosphorylation, Denton *et al.* report in another communication (*Proc. Biochem. Soc.*, in the press, 1971) the lack of a direct effect of cyclic AMP on the dehydrogenase enzyme. Further work on the mechanism by which insulin stimulates pyruvate dehydrogenase will now be awaited with great interest.

FISH

The Disappearing Goby

from our Marine Vertebrate Correspondent

GIVEN a river estuary in which population of fishes is extremely large from July to January when the fishes' gonads are reaching maturity and then almost disappears, which explanation is most likely to explain the disappearance of the fish—offshore migration to breed; low temperatures; lowered salinity; lack of food; or predation? In a study on the distribution and abundance of the sand goby (*Gobius minutus*, better known in recent literature as *Pomatoschistus minutus*) in the estuary of the Ythan, just north of Aberdeen, M. C. Healey (*J. Zool.*, **163**, 177; 1971) attempts to answer these questions.



Sand goby

In the Ythan estuary the sand goby is abundant from July when the young of the year enter the estuary for the first time and the class of the previous year return to the river. After recruitment stops, losses are gradual until January or February when about 85 per cent of the first year fish disappear. A second decline in the population occurs the following November when the fish are in their second year and the year class is almost extinguished. Because the January–February decline occurred when the gonads of both sexes were mature or close to maturity it seemed quite natural to assume that the disappearance was connected with breeding behaviour, and this seemed to be confirmed by the sex-ratio which changed from unity during the period of abundance to about one male to five females after February.

Healey has attempted to explain these observations by a series of alternative hypotheses. First, he considers the possibility that the gobies cannot obtain sufficient food in the spring months, hence they migrate or die off. His observations show that the most important item in their diet is the amphipod crustacean, *Corophium volutator*, and taking into account the daily requirements of the goby population, he demonstrates that there can be no shortage of food.

Another possible explanation is that the sand goby migrates from shallow inshore waters to escape low winter temperatures, and this has been suggested by other workers. Healey, however, dismisses this on the grounds that in the Ythan temperatures are rising by February when the migration begins. Similarly, lowered salinity is

ruled out as a causative factor because Healey could find no regular seasonal change in salinity in the area.

The possibility that the gobies were simply migrating offshore to breed is also considered. *Gobius minutus* is territorial in its breeding season; the males build and guard "nests" under empty bivalve shells. Consequently the population tends to spread out over the sea bed to a random pattern in response to the availability of suitable ground. The fact that males seem to decline in the catches in February could well be associated with their spreading out and becoming harder to catch in their shell "nests".

Although other workers have found that the scarcity of the sand goby in inshore waters is matched by an increase offshore, Healey's attempt to catch them offshore was not successful. It is curious, however, that in contrast to the wealth of data on density, fishing effort, and net efficiency for the estuary work, Healey is remarkably coy about details of the offshore fishing effort. It is said that offshore fishing was not intensive, but the only data given seem to amount to a total of four hours and forty minutes trawling on six separate days, nothing about the size of the net, its mesh, or the speed at which it was hauled. On this evidence Healey concludes that the sand gobies had not moved offshore to spawn, and he therefore advances another hypothesis that the activities associated with breeding

expose the fish to a high level of predation. As he observes, there are a number of fish and fish-eating birds in the estuary which might prey on gobies if their behaviour made them available. It seems remarkable that after two useful years' work on the biology of the sand goby Healey, in an article of more than fifty pages containing twenty-one tables and fourteen text figures, can write in support of this predator hypothesis "since I know nothing about the diets of various predators at the important time of the year, it is a completely speculative hypothesis".

PHOTOSYNTHESIS

Break in the Chain

from our Photosynthesis Correspondent

IT seems likely that the b-type cytochrome (cyt b_{559}) found in the chloroplasts of green leaves is not located on the principal electron transport pathway linking photosystem two (S2) with photosystem one (S1). The occurrence of cyt b_{559} in chloroplasts is well known but its place in the photosynthetic electron transport chain is far from certain. In fact, at room temperature there is no observable photo-oxidation or photo-reduction of this cytochrome *in vivo*. Recently, however, Boardman and his colleagues in Canberra have presented evidence which indicates a possible site

Measuring Coronal Line Profiles

IN the forthcoming issue of *Nature Physical Science*, G. Henderson, A. Larsen and P. M. Marshall of Heriot-Watt University describe the latest stage of the development of the Fabry-Perot method for the investigation of solar coronal line profiles.

Henderson and his colleagues have used their Fabry-Perot spectrometer in conjunction with the coronagraphs at the Pic du Midi Observatory in the Hautes Pyrénées and at Wendelstein, Ober Bayern. The essential features of any coronagraph resemble those of an astronomical telescope except that the glass of the objective lens must be absolutely flawless and completely clean so that there is as little scattering of the sunlight as possible. In essence, most coronagraphs produce an artificial solar eclipse by the introduction of an occulting disk in an appropriate position so that only the corona itself is observed.

The Heriot-Watt team have made line profile measurements on the 5303 Å (FeXIV) and 6374 Å (FeX) lines of the solar corona by focusing the coronal region of interest onto the entrance aperture of the Fabry-Perot spectrometer. The coronagraph telescope al-

ways points at the centre of the Sun and so a pair of gimbaled mirrors had to be positioned between the final lens of the coronagraph and the entrance aperture of the spectrometer. In order to scan through the wavelength range of interest, the separation of the Fabry-Perot reflecting plates is modulated by means of piezoelectric crystals. Finally, the light emerging from the reflecting plates is passed through a narrow band interference filter and onto a photomultiplier tube which records the variation of intensity with wavelength.

During a four week period in October 1970, successful observations were made at the Pic du Midi and a total of 1,200 line profiles of the red and green lines were collected from the coronal region as far out as $1.6 R_{\odot}$ from the solar limb. Weather conditions at Wendelstein were unfortunately not very good during this period and only ten profile measurements of the green line at $1.1 R_{\odot}$ were possible. The large amount of potential data now available on coronal line profiles should enhance knowledge of the environment and energy distribution of the highly ionized atoms which populate the corona.

for this cytochrome (*Biochim. Biophys. Acta*, **234**, 126; 1971). They have carefully analysed light minus dark difference spectra measured over the wavelength region of 500 to 600 nm on intact leaves and isolated chloroplasts at 77 K. At this temperature the spectra obtained are due to electron transfer between donors and acceptors which are in very close proximity.

Boardman *et al.* have confirmed the observation of Knaff and Arnon (*Proc. US Nat. Acad. Sci.*, **64**, 715; 1969) that cyt b_{559} is oxidized at 77 K by light primarily absorbed by S2 and have presented further evidence that this photo-oxidation only occurs when the primary electron acceptor of S2 (called E by Boardman) is not reduced. Therefore it seems that cyt b_{559} donates electrons to, rather than, as originally thought, accepting them from, E. If this is so, then it could mean that cyt b_{559} functions as a carrier for cyclic electron flow.

In the same article it is convincingly shown that cyt f , the c-type cytochrome which is also found in chloroplasts, is not photo-oxidized at 77 K. In the past it has been reported that this cytochrome is photo-oxidized by S1 light both at room and liquid nitrogen temperatures (B. Chance and W. D. Bonner, in *Photosynthetic Mechanisms in Green Plants*: Nat. Acad. Sci.—Nat. Res. Council Pub. No. 1145, 66; 1963). Because of this it had been concluded that not only did cyt f lie on the principal electron transport pathway between the two photo-systems but that it was in intimate contact with the reaction centre chlorophyll of S1. The confusion whether or not cyt f is photo-oxidized at low temperature apparently arose because of a misinterpretation of the bands which occur at 547 nm and 557 nm in the light minus dark difference spectra at 77 K. It is now well established that the 557 nm absorbancy change is due entirely to the photo-oxidation of cyt b_{559} and the 547 nm band has clearly been shown by Boardman's group not to correspond to the 548 nm band associated with cyt f oxidation. In fact, the Canberra group have confirmed Arnon's proposal that the low temperature light induced spectral change at 547 nm is due to a primary photoreduction. The nature of the photoreduced compound is unknown but could be the primary electron acceptor of S2 (E).

The removal of cyt b_{559} from the electron transport chain linking S2 and S1 leaves an embarrassing gap in the well established "Z" scheme. This can only mean that students of photosynthesis will have to accept yet another letter of the alphabet to signify a hypothetical intermediate which acts on the reducing side of E.

MAN-MADE LAKES

Problems Defined

from a Correspondent

A BROADLY based interdisciplinary symposium on the problems and environmental effects of man-made lakes was held at the University of Tennessee, Knoxville, Tennessee, from May 3 to 7, under the auspices of the Scientific Committee on Water Research (COWAR) and the International Council of Scientific Unions (ICSU).

It was immediately apparent that there is a need for a register of man-made lakes of the world. Dr R. Keller (University of Freiburg) suggested that such a register be compiled by one of the UNESCO organizations to complement the *World Register of Dams*. The latter publication gives engineering details of about 10,000 dams but provides few specifications concerning the dammed water bodies.

The importance of preimpoundment studies in relation to public health, population resettlement and other socio-economic factors was stressed by several contributors. Dr E. A. K. Kalitsi (Volta River Authority, Ghana) considered the Volta Lake as one of the major instruments for transforming the Ghanaian economy. The formation of the lake had created new economic possibilities but it had not been possible to exploit fully the opportunities offered. Failure to complete land

acquisition and land clearance before resettlement had led to a lowering rather than an increase in production. Similarly, failure to implement fully irrigation programmes, the development of lake fisheries, transport and tourism has given rise to unrealized expectations.

Dr A. W. A. Brown (World Health Organization) considered the public health problems created by man-made lakes. In tropical and subtropical man-made lakes, notably those in Africa, explosive outgrowths of water weeds after filling had led to a very significant increase in the incidence of diseases carried by insects, such as malaria and oncocerciasis, and by molluscs such as schistosomiasis. The need for containment of disease vectors and for adequate environmental sanitation was emphasized, and it was suggested that a centre should be set up to study these problems.

In the field of limnology Professor B. R. Allanson (Rhodes University) stressed the need for continued fundamental research by multidisciplinary teams. He considered that appreciation of eutrophication might well be hampered by the continuing tendency to work within too rigid a framework of parameters. He suggested that although estimation of the elements nitrogen and phosphorus should not be discontinued there should be more emphasis on measurements of carbon.

Specificity of Antibody Responses

HETEROGENEITY among mouse lymphocyte populations which are morphologically very similar is a source of sadness to the descriptive cytologist and a great joy to the experimental cellular immunologist. At least two sorts of cells, named T and B to indicate their derivation from thymus and bone marrow respectively, are widely recognized. Numerous studies in which the derivation of cells has been determined have established this point beyond question.

But, aside from these organizational findings, many other workers have shown that B cells produce antibody whereas T cells act in a cooperative role in antibody production, being apparently incapable of antibody synthesis themselves. Thus the heterogeneity is related not only to cell surface antigenicity and derivation but also to a functional attribute. In next week's *Nature New Biology*, Basten *et al.* show that the specificity of antibody responses in which cooperation between B and T cells has occurred is apparently determined by both of the cellular constituents of the reacting mixture.

The method adopted was to incubate either T or B cells (thymocytes or cells

from the spleens of thymectomized irradiated bone-marrow injected mice) with a protein which had previously been bound to ^{125}I . The incubated cells were then transferred into irradiated mice which were challenged with the same protein (not iodinated) or a control antigen. The theory was that the "hot" antigen would bind to and destroy those cells which normally react to the protein. The results indicated that a specific reduction of twenty to thirty-fold in the numbers of antibody releasing cells, as adjudged by a plaque assay, was evident when either T or B cells were incubated with the hot antigen. From a further series of experiments it seems that the receptor on the thymocytes for the protein antigen is an immunoglobulin or is linked with an immunoglobulin on the cell surface.

For the connoisseur it should be noted that suicide experiments with B cells present in bone marrow inocula failed. Basten *et al.*'s suggestion that there may well be few mature B cells in mouse bone marrow, implying therefore that this tissue is not a particularly useful source of B cells in cooperation experiments, may not be received kindly.

TUMOUR VIRUSES

Pulling Together

from a Correspondent

To mark the sixty-fifth birthday and retirement of their director, Professor O. Mühlbock, the Netherlands Cancer Institute organized in Amsterdam last week (May 12–15) a verbal *Festschrift*—a conference on RNA tumour viruses and oncogenesis with particular emphasis on the role of the host genome in the response of an animal to tumour viruses. And for once the murine mammary tumour viruses were as fully discussed as the avian and murine sarcoma leukaemia virus complexes; there are, of course, more virologists—most of them Mühlbock's pupils—studying mouse mammary tumour viruses in Holland than in any other country with the possible exception of the United States.

Once the stage had been set by a series of speakers who surveyed the history of the subject, the conference got down to first discussing the genetic factors influencing oncogenesis by avian viruses. Svoboda (Czechoslovakia) summarized a series of experiments showing that Rous sarcoma virus (RSV) can be rescued from transformed mammalian cells by fusion with chick fibroblasts if the transformed cells carry the group specific antigens of the avian tumour viruses. By contrast, if the transformed mammalian cells lack the group specific antigens, RSV cannot be rescued by fusion with chick fibroblasts.

Payne (UK) then reviewed the elegant genetic analysis of the control of susceptibility of chicks to avian sarcoma and leukaemia viruses which have won him and his colleagues renown. Susceptibility to viruses of subgroups A, B, and C is controlled by three corresponding loci, the allele for susceptibility being dominant to that for resistance. Susceptibility to viruses of subgroup E—the new designation of the Bryan high titre sarcoma virus genome and RAV.60—is, however, more complex; in addition to a locus with dominant susceptibility and recessive resistance alleles there is a second epistatic inhibitor locus. The dominant and widespread allele of this inhibitor locus (I^0) renders cells resistant to E subgroup viruses even when the cell is homozygous for the susceptibility allele.

Weiss (UK) then reported fascinating experiments which show that all chick cells carrying the group specific antigens of the avian viruses can provide a helper function for RSV.E, and chick factor, otherwise called RAV.0 or RAV.60, can be obtained from these cells. Chemical carcinogens and ultraviolet and X-irradiation all promote this activation and release of the resident RAV.60 or RAV.0 leukosis virus.

Furthermore, even cells which lack detectable group specific antigens may act as helper for RSV.E and occasionally leukosis virus seems to be rescuable from these cells. Hanafusa (USA) reported essentially similar results and conclusions as Weiss. It is beginning to look as if all chick cells, even those of wild Malaysian jungle fowl, carry the leukosis virus RAV.60 (or RAV.0).

The Dutch school of murine mammary tumour virologists have come to much the same conclusion about their system. Hageman and Bentvelzen (Holland) suggest that no strain of mice is free of mouse mammary tumour virus (MMTV), and Bentvelzen's impressive experiments clearly indicate that several strains of MMTV are transmitted vertically by egg and sperm rather than in milk. He believes that every mouse carries the genes to specify an MMTV, and that the development of mammary carcinoma depends on the "derepression" of these genes and their replication as a virus.

Whether mammary carcinoma develops depends on hormonal factors (Boot, Holland) and on the host's genotype. Dux and Mühlbock (Holland) reported that alleles of the H2 histocompatibility locus play an important part in regulating the response of an animal to strains of MMTV; the H2b allele, for example, inhibits oncogenesis by C3H-MTV (Bittner agent).

Boyse (USA) and Lilly (USA) described the striking parallels between the influence of histocompatibility antigens on the response to MMTV and murine leukaemia viruses. As Boyse so

eloquently explained, the H2 locus, the TLA locus and the G9 locus, all part of the murine linkage group IX, are implicated in murine leukaemogenesis. Leukaemic cells of strains of animals which are, when healthy, TLA⁻ and G9⁻ carry at least two TL antigens and the G9 antigen. These loci seem to be switched on by leukaemogenesis, and although not committing himself Boyse raised the possibility that the G9 locus might be part of a murine leukaemia virus genome present in all mice. Murine leukaemogenesis might well boil down to the activation of a latent murine leukaemia virus genome transmitted vertically in mice which in the environment of the thymus might transform target lymphoid cells.

On the final morning Spiegelman (USA) reviewed the state of play with reverse transcriptase, noted in passing that visna and slow pneumonia viruses have now been shown to transform cells *in vitro*, reported that the B-type particles (or are they C-type?) in human milk contain reverse transcriptase, and left the stage for Temin (USA) to expound his provirus theory. Temin argued that gene amplification may be important in normal differentiation and may be mediated by messenger RNA and reverse transcriptase. Cell transformation might result from the amplification of transforming genes either introduced by a transforming virus or resident but not normally expressed in a differentiated cell but induced to amplify by the virus. His was a thought provoking climax to a noteworthy conference.

New Soft X-Ray Source Discovered

As well as the exciting developments from the experiments on board the Uhuru X-ray satellite, the by now conventional sounding rocket techniques continue to add to the list of known X-ray sources. Below 2 keV, the lower limit of the sensitivity of Uhuru's detectors, sounding rockets remain the prime source of experimental data. The latest development in this field is the discovery of a soft X-ray source in the Aries-Taurus region. This discovery was made by the large team of high energy astronomers working at Nagoya University; their full report of the discovery will be published next week in *Nature Physical Science*.

At 0.2–0.5 keV, the source is brighter than NGC 1275, the Seyfert galaxy recently found to be an X-ray source, which appears in the same part of the sky. But at higher energies the relative brightness of the new source decreases until it can no longer be detected above 1 keV. NGC 1275 is still detectable at the higher energy range, and this points, not unexpectedly,

to a different mechanism operating in the new source. The statistics available so far do not really allow any definite estimate of the source's spectral characteristics, but it is a very soft source, and the spectrum is at least not inconsistent with the familiar exponential form with kT between 0.3 and 0.5 keV. A lower limit on the total energy flux from the source in the observed range is roughly 2×10^{-9} erg cm⁻² s⁻¹.

Comparison with Sco X-1, the brightest known X-ray source within our galaxy, shows just how wide the classification "X-ray source" is—Sco X-1 has a very hard X-ray tail to its spectrum (above 40 keV), as well as a softer, varying component at 1–10 keV, and if the energy mechanism is thermal the effective temperature in the source is roughly 50 million K. Indeed, it is now little more help to say that an object is an X-ray source than to say that it is a star. With the further development of high energy astronomy, more specific classifications will undoubtedly become clear.

Role of Subunits in the Ribosome Cycle

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In this article Dr Davis reviews recent developments in the search for the precise nature of the cycle of ribosomes in protein synthesis.

UNLIKE many aspects of protein synthesis, the relations of free ribosomes and subunits to the ribosome cycle remained obscure for a long time. Early studies¹ showed that at low divalent ion concentrations (for example, 0.1–0.3 mM Mg^{2+}) the 70S bacterial ribosome dissociates reversibly into a large (50S) and a small (30S) subunit; and the 80S ribosomes of eukaryotic cells dissociate similarly, at still lower Mg^{2+} concentrations². Moreover, in lysates with adequate Mg^{2+} concentrations at least 10% of the ribosomal particles appear regularly as “native” subunits³. However, the “derived” subunits observed at low Mg^{2+} are obviously artefacts, formed in conditions well outside the range required for protein synthesis; and the “native” subunits might be damaged or immature, for they are not associated with a nascent polypeptide chain and they do not form 70S ribosomes at increased Mg^{2+} concentrations. Nevertheless, it seemed curious that ribosomes are regularly constructed of such a loosely complexed pair of subunits.

Tissières *et al.* suggested in 1960 that ribosomes might normally split into subunits, after release of the growing peptide⁴, when they found that “active” ribosomes carrying nascent polypeptide are harder to dissociate than inactive ribosomes. Watson⁵, summing up the state of progress in 1964, concluded that “some cycle must exist which demands the coming apart of the two subunits. It is thus very important to know the proportion of subunits which exist free during protein synthesis”. Indeed, measurements of subunit levels eventually proved to be crucial. But the appropriate part of the cycle, the initiation step, was ignored for several years: other aspects of protein synthesis could be studied profitably with synthetic messengers; and the high Mg^{2+} concentrations used in these systems permit non-specific initiation (apparently on the 70S ribosome), bypassing the natural specific initiation step on a subunit.

Moreover, after the RNA from bacterial viruses (MS2, R17 and so on) was introduced as a natural messenger⁶, physiological initiation was found to be a complex process^{7–9}. At an initiating site on mRNA the codon AUG codes for a special initiating tRNA, formylmethionyl-tRNA (fMet-tRNA)¹⁰; binding of this ligand involves ribosomal subunits and several specific protein factors; and one of these factors regulates the formation of subunits in the ribosome cycle.

This article reviews the evidence on subunit formation and function.

Evidence for Initiation by Subunits

The first convincing evidence for physiological dissociation of the ribosome was provided in 1968 by Kaempfer, Meselson and Raskas¹¹: when *Escherichia coli* cells were grown first with and then without heavy label (2H , ^{13}C , ^{15}N), the subunits were either heavy or light, but the ribosomes included particles of intermediate density. These must have cycled through a pool of subunits and exchanged partners. Closer kinetic analysis, with protein-synthesizing extracts, further suggested that the ribosome undergoes subunit exchange in each round of translation^{12,13}.

This approach provided unequivocal evidence for dissociation of ribosomes during the ribosome-polysome cycle. The specific function of the resulting subunits was meanwhile revealed by Nomura^{14–16}, who showed that with viral RNA as messenger the small subunits bound the initiating fMet-tRNA more effectively than did 70S ribosomes. The basic mechanism that was inferred has been extensively confirmed^{7–9}. mRNA and fMet-tRNA bind first to a small subunit to form a “30S” initiation complex (which may, of course, be on a polysome); this complex is then joined by a large subunit to form a completed “70S” initiation complex; and when the latter transfers the fMet to the next aminoacyl-tRNA it becomes a polypeptide-bearing, “polysomal” ribosome.

Initiation Factors

The function of the subunits is intertwined with another aspect of the process of initiation—the involvement of several special protein factors—that had been discovered even earlier. Ochoa¹⁷, Revel and Gros¹⁸, and Eisenstadt and Brawerman^{19,20} showed that ribosomes washed with 0.5 M or 1.0 M NH_4Cl had lost their activity with viral RNA as messenger, but not with poly U; and full activity was restored by adding the proteins that had been extracted. Fractionation of the extract on DEAE-cellulose eventually yielded three required components: F_1 , F_2 and F_3 of Ochoa²¹, corresponding to Gros’s A, C and B²², respectively. In the usual conditions factor F_1 (A) passes through DEAE-cellulose, F_2 (C) is strongly adsorbed, and F_3 (B) is weakly retained. The identification of F_3 has sometimes been confusing, for it comes out partly or entirely with F_1 if the sample has a slightly increased salt concentration or contains urea, or if the column is heavily loaded^{23,24}.

All three factors have been highly purified. F_1 has a molecular weight of 9,000 (ref. 25), F_2 70,000 (refs. 26 and 27) and F_3 21,200 (ref. 28). F_1 and F_2 promote the firm binding

of fMet-tRNA, accompanied by the hydrolysis of GTP^{21,22,25,26}. The detailed interactions, however, are not altogether clear. Evidence has been presented both for²⁸ and against²¹ an additional function of F₂ in promoting the binding of natural mRNA. I shall discuss the function of F₃ later in this article.

In contrast to the factors involved in chain extension (T_u, T_s and G)³⁰ and in polypeptide release (R₁ and R₂)^{31,32}, the initiation factors are found in the ribosomal pellet rather than in the supernatant in a sedimented cell lysate. Moreover, they have a special distribution in the pellet: key studies of Eisenstadt (refs. 19, 20 and 33; but see also 18, 34) showed that the initiation factors are present only in the native 30S and not in the native 50S or the 70S fraction of *E. coli*. Thus these factors are not constant components of the ribosome, present at all stages of its cycle; rather, they must have a cycle of their own, attaching to the small subunit and being released at some stage in its subsequent conversion into a

The early bacterial lysates, prepared by grinding with alumina, contained only 70S ribosomes and subunits. Gentler disruption of the cell, by digesting its wall with lysozyme^{40,41}, later yielded lysates containing not only 70S ribosomes and subunits but also a high level (> 60%) of polysomes, chains of mRNA carrying a number of active ribosomes. Mangiarotti and Schlessinger⁴², however, developed a method of lysis that yielded only polysomes and subunits. On the assumption that the method yielding the fewest 70S particles was the gentlest, they concluded that the 70S particles obtained by other "gentle" methods resulted from artificial fragmentation of some polysomes during lysis, which converted them into complexed 70S ribosomes (that is, ribosomes carrying peptidyl-tRNA and a short length of messenger). Because the cell thus appeared to lack 70S ribosomes, it was also reasonable to conclude that a ribosome released at the end of a round of translation immediately and spontaneously dissociates into a pair of subunits.

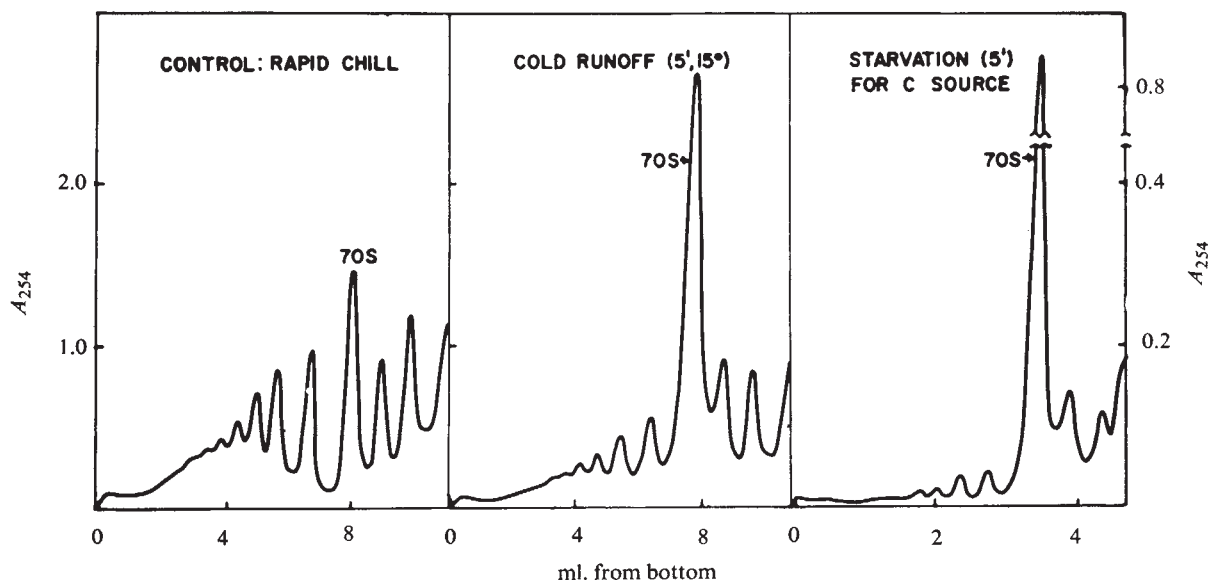


Fig. 1 Conversion of polysomes to 70S ribosomes by two mechanisms of runoff. Growing cells of *E. coli* were treated as indicated and then lysed by freeze-thaw lysozyme-deoxycholate in TKM buffer (10 mM Tris, 50 mM KCl, 5 mM Mg²⁺) with chloramphenicol and DNAase, and the lysates were analysed by sedimentation in a sucrose gradient containing TKM. (The apparent decrease in the absolute level of subunits after runoff is due to the analysis of a smaller sample.) (From ref. 54.)

polysomal ribosome. This release has been demonstrated directly with radioactively labelled F₁ (ref. 35). It should be emphasized that such a direct or indirect demonstration of cyclic attachment and detachment is essential for establishing a factor: for otherwise a protein that restores the activity of the ribosomes from which it has been extracted³⁶ could be either a factor or a loosely bound true ribosomal protein.

The distribution of the initiation factors also indicates that at least some of the native subunits are active. This fraction would thus be rapidly turning over. Indeed, it had been noted earlier that growing cells incorporated labelled precursors as rapidly into the subunit fraction as into polysomal ribosomes³⁷; but with the method of lysis used the subunits observed would include products of artificial dissociation of the free ribosomes³⁸, as well as native subunits.

Production of Free 70S Ribosomes

In contrast to the function of the subunits in initiation, the mechanism of their formation still seems to be controversial^{8,9,28,39}. I shall therefore review the evidence in some detail.

A major problem has been the preparation of lysates that might reveal the distribution of ribosomal particles in the cell.

These findings received wide attention, for they provided the first evidence for initiation of protein synthesis by subunits, which was later directly demonstrated, as I have noted. But though absence of free ribosomes would imply initiation by subunits, the reverse is not true. Moreover, the evidence for their absence was not conclusive. The lysates that lacked 70S ribosomes⁴² had a rather high level of subunits, which suggested that the method used might have caused an increase in ribosome dissociation rather than a decrease in polysome fragmentation. Moreover, earlier work had suggested that runoff ribosomes might be especially susceptible to dissociation: for in alumina-prepared lysates synthesizing labelled polypeptide only a fraction of the 70S particles were engaged in synthesis, and these were less easily dissociated than the inactive particles as the Mg²⁺ concentration was decreased⁴.

The proposed complete dissociation of ribosomes after their runoff from polysomes could be critically tested by identifying the products of an increase in net runoff. To achieve this increase several methods were used: treatment with actinomycin, treatment with puromycin, starvation for a carbon source or deprivation of a required amino-acid. The resulting decrease in polysomes was uniformly accompanied³⁸ by an increase in the 70S peak rather than in the subunits (Fig. 1). Other

laboratories obtained similar results after increasing the runoff by deprival of a required pyrimidine⁴³ and by slow cooling of the cells⁴⁴. Since the diverse methods used to cause runoff could hardly share a common artefact, it seems clear that the products of runoff accumulate in the cell as 70S ribosomes. I shall discuss later in this article the question of whether these particles are immediate or secondary products of runoff.

The various methods used to cause accumulation of runoff ribosomes strongly suggested that they lack nascent polypeptide (or fMet) and mRNA, and this inference was supported by observations with radioactive labelling^{38,44}. Moreover, we have demonstrated 70S runoff ribosomes that lack tRNA (unpublished results of P.-C. Tai and myself). It therefore seems clear that runoff can yield truly free 70S ribosomes.

Dissociability of Ribosomes

With lysates known to contain predominantly complexed ribosomes (that is, polysomes fragmented by RNAase) or predominantly free (that is, runoff) ribosomes, the postulated difference in their dissociability could be demonstrated by zonal centrifugation in the presence of 1 mM Mg^{2+} (refs. 45 and 46). This test made it possible to show that free ribosomes are not peculiar to conditions of impaired protein synthesis: they are also present in the small steady state 70S peak in lysates of growing cells⁴⁵. (The method that had failed to yield such a peak⁴² was shown³⁸ to promote dissociation.) That free ribosomes are dissociated more easily than complexed ribosomes is hardly surprising: the peptidyl-tRNA on the latter presumably forms a bridge between mRNA (known to bind to the 30S moiety) and the peptidyl transferase (known to be part of the 50S moiety).

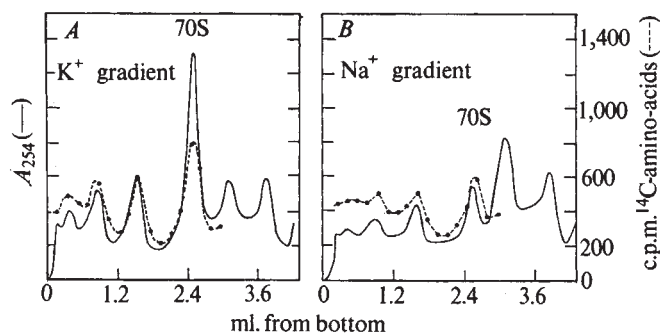


Fig. 2 Presence of Na^+ instead of K^+ in buffer in sucrose gradients causes dissociation of free 70S ribosomes but not of polysomes or of 70S ribosomes carrying nascent polypeptide. Cells were pulse-labelled with ^{14}C -amino-acids before lysis. (From ref. 49.)

It thus seemed clear that the 70S particles observed in lysates cannot be entirely accounted for by polysome fragmentation. But another way to account for these particles as artefacts was proposed by Phillips *et al.*^{47,48}, who found that growing cells yielded a substantial 70S peak when lysed in a buffer containing 60 mM K^+ but not in buffer containing Na^+ instead. They favoured the interpretation that runoff yields only subunits, and that K^+ , unlike the unphysiological ion Na^+ , allows these to form 70S initiation complexes during preparation of the lysate. However, the differential effects of K^+ and Na^+ on the ribosomal profiles have now been traced to quite a different mechanism: the selective dissociation of free ribosomes by Na^+ (ref. 49). As Fig. 2 shows, a gradient containing Na^+ dissociates those 70S ribosomes that do not carry nascent polypeptide but leaves complexed ribosomes intact.

The "normal", steady state 70S peak in growing cells is thus a mixture of free and complexed ribosomes. (Earlier studies, which eliminated the 70S peak entirely while preserving

the polysomes^{42,45}, used analytical methods too crude to detect a small residue of complexed ribosomes.) Moreover, small variations in procedure (including the rate of chilling of the cells) markedly affect the ratio of the two major classes of 70S ribosomes^{49,50}. This effect no doubt has contributed to the differences in ribosomal profiles observed in different laboratories.

The differential dissociability of free and complexed ribosomes has given rise to another effect, which has only recently been understood. In several laboratories free ribosomes, but not complexed ribosomes, were observed to sediment at 60S–65S at high speeds, or at salt concentrations that nearly cause dissociation, and a loosening of ribosome conformation was inferred. Infante, however^{51,52}, has shown recently that a high hydrostatic pressure in the centrifuge tube promotes ribosome dissociation. Thus in a given ionic environment, and at a given centrifugal speed, the ribosomes will dissociate at a particular position in the tube; then for some distance, before the newly formed subunit peaks are resolved, they will appear to sediment as a "slow" ribosomal peak.

Ribosome Dissociation Factor

The study of ribosome runoff³⁸ showed that only the 70S ribosomes increase as the polysomes decrease: within experimental limits the level of subunits does not rise at all (Fig. 1). This finding suggested that runoff ribosomes are not in spontaneous equilibrium with native subunits: rather, a runoff ribosome must be converted to subunits in the cell only by stoichiometric complexing with a ribosome dissociation factor (DF), the limited supply of which thus regulates the level of the subunit pool. Moreover, because NH_4Cl -washed ribosomes can initiate effectively when supplemented with the three known initiation factors, and because this process presumably requires dissociation, it seemed likely that the initiation factors would include DF.

A crude initiation factor preparation from *E. coli* (that is, a 1 M NH_4Cl extract of the ribosomal pellet or of its 30S fraction) was indeed found to dissociate runoff ribosomes⁵³, but not complexed ribosomes⁵⁴ (Fig. 3). This activity has been confirmed, not only with runoff ribosomes⁴⁴, but also with ribosomes washed with 1 M NH_4Cl ^{24,28,55}, which evidently removes all stabilizing ligands. The reaction is essentially complete within a minute in dilute solution at 37° C (but is very slow at 0°)⁵³; it is probably much faster at the 200-fold greater ribosome concentration in cells.

DF is Initiation Factor F_3

The relation of DF to the initiation factors was studied with purified materials given by Professor S. Ochoa⁵⁶. F_1 and F_2 had no activity, but F_3 was highly active⁵⁴. Definitive identification has recently been achieved^{24,28} with a preparation of F_3 exhibiting a single band on acrylamide gel electrophoresis²⁸.

Though DF dissociates only about 1/20 its molar equivalent of ribosomes at 4 mM Mg^{2+} , the ratio increases to 1/2 at the lowest Mg^{2+} concentration (1.5 mM) that prevents spontaneous dissociation²⁴. This value, and the shape of the concentration-activity curves, support a first-order reaction. Further support is provided by the calculation²⁴ that the amount of DF recovered per cell²⁸ is in the proper range for one molecule per native small subunit. The stoichiometry at low Mg^{2+} also excludes the possibility that the DF activity of the F_3 preparation could reside in a minor component.

The relatively low apparent affinity of DF for the ribosome fits its cyclical function, in which it is bound at one stage and displaced at another. The assumed reversible equilibrium between DF and ribosomes, however, has not been established: for though subunits freshly produced by DF can reassociate at higher Mg^{2+} (ref. 54), and can exchange with free ribosomes (unpublished results of R. J. Beller and myself), investigation of the equilibrium has been hampered because isolated subunits readily lose these reactivities.

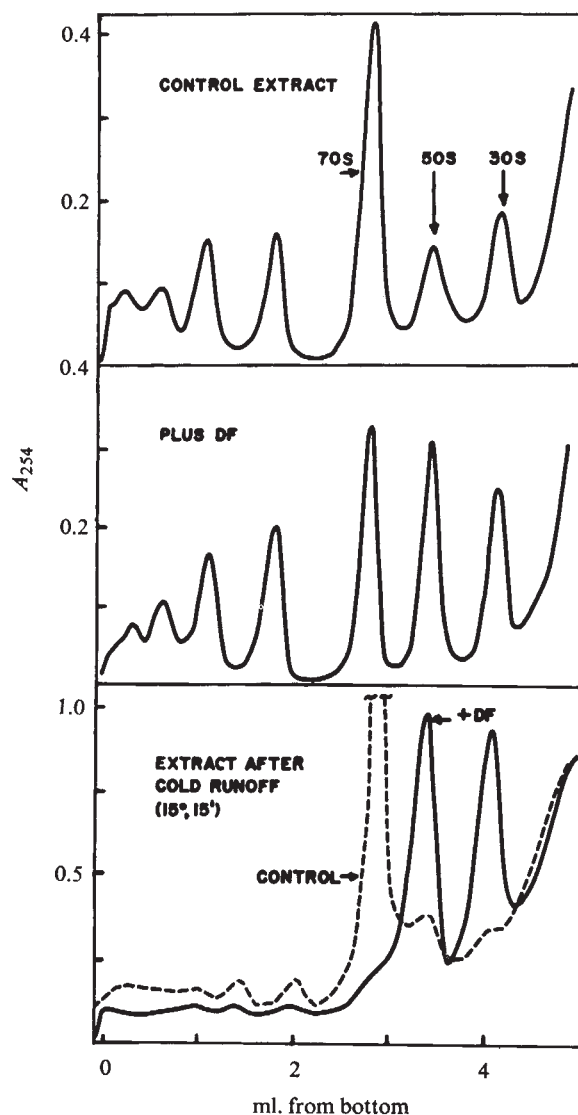


Fig. 3 Excess of DF dissociates all runoff ribosomes and part of the normal 70S peak, but not polysomes. Lysates were incubated with same amount of purified DF for 5 min at 30° C before analysis. (From ref. 54.)

F_3 not only decreases the affinity of the small subunit for the large subunit but also seems to increase its affinity for mRNA. Thus in the presence of the other two factors F_3 stimulates 30S initiation complex formation with viral RNA as messenger but not with trinucleotide AUG²⁸. (Similar observations with 70S ribosomes had earlier suggested the same conclusion⁵⁷, but the effect could be due to the conversion to subunits by F_3 , discovered later.) F_3 thus seems to participate in the recognition of an initiation sequence, which must be longer than AUG⁵⁸. Moreover, the specificity of this recognition may be important in regulation: for in cells infected with phage T4 the preference of the ribosomes for different messengers is altered⁵⁹, apparently through an alteration in F_3 (ref. 60).

With the identification of DF with F_3 the former term could perhaps be abandoned. However, a descriptive designation for a biochemical component seems preferable to an arbitrary symbol, and the initiation factors presently bear the additional burden of three competing arbitrary terminologies^{17,18,61}; thus it may be desirable to retain the term dissociation factor until a definitive terminology for all three initiation factors is agreed on.

The Ribosome Cycle

The findings reviewed here suggest the ribosome-polysome cycle and the interlocking DF cycle, depicted in Fig. 4. In

this model ribosomes are released from mRNA as 70S particles. If, however, as seems reasonable, the elaborate mechanism of initiation by subunits serves to provide a ring-like binding of the ribosome on the mRNA, permitting motion along the chain without premature falloff, then later release at a termination signal might well also require dissociation, to permit opening of the ring. The observed free 70S ribosomes would then be secondary rather than immediate products of runoff. Indeed, when Kaempfer³⁹ recently allowed polysomes labelled with heavy isotopes to run off *in vitro* together with "light" polysomes the resulting 70S ribosomes had all exchanged subunits. From these and related observations he concluded that runoff yields subunits as its immediate product, and these form stable 70S ribosomes only when they are prevented from rapidly reinitiating.

In the absence of kinetic studies, however, it is not certain whether the observed exchange had occurred during or after runoff. Because the presumably reversible action of DF might be expected to promote exchange without involving a pool of runoff subunits, Subramanian (unpublished results) repeated this key experiment but allowed the "heavy" and "light" polysomes to run off separately. When the resulting solutions of free ribosomes were mixed, subunit exchange was also complete.

It thus seems that free ribosomes are in rapid equilibrium, under physiological ionic conditions, with a very low concentration of free subunits. This conclusion has been independently reached by Infante and Baierlein⁵² on the basis of quite a different kind of observation: the sedimentation patterns obtained at high hydrostatic pressures conform to a rapid equilibration, with elevated pressure shifting the equilibrium constant in the direction of increased dissociation. With runoff thus yielding a rapidly equilibrating mixture, it may be difficult to determine whether or not ribosome release requires dissociation.

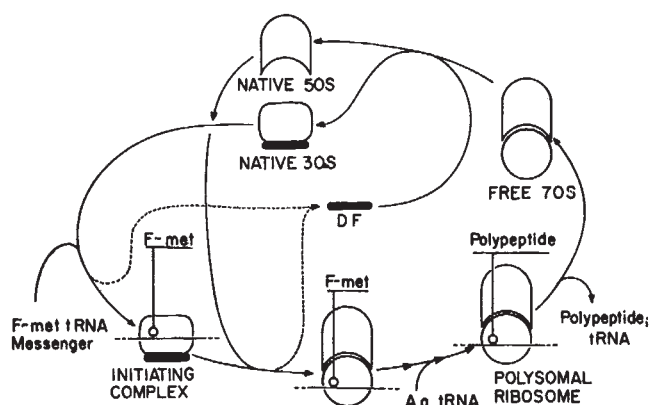


Fig. 4 Proposed role for DF (F_3) in the ribosome-polysome cycle. The other two initiation factors also become part of the native 30S subunit and are released in the course of its incorporation into a polysomal ribosome, but the precise sequence of these interactions is not known. (From ref. 53.)

This equilibration also suggests a possible modification in the model presented in Fig. 4. DF might still produce stabilized subunits by attacking a 70S ribosome, as in that model; but it might alternatively interact only with the trace of free small subunits, increasing the net dissociation by shifting the equilibrium.

To account for the evolutionary advantage of a mechanism of dissociation involving DF it has been suggested⁵³ that optimal flow in the cycle of dissociation and reassociation probably requires an appropriate level of the initiating subunits.

With DF regulating this level a change in the rate of protein synthesis would alter the size of the ribosome pool rather than that of the subunit pool.

Though initiation by subunits can account for the construction of the ribosome from two loosely joined moieties, evolution might well take further advantage of this structure. For example, in chain extension the translocation step requires both peptidyl-tRNA and mRNA to move from one position (A) to another (P) on the ribosome, without losing their firm attachment. Bretscher⁶² has suggested that these requirements might be met by a two-step model, in which each subunit in turn rotates a little relative to the other: the ligands could thus retain a firm purchase on one subunit while moving on the other. Spirin⁶³ has suggested a model with movement roughly perpendicular to this one, in which aminoacyl-tRNA would be attached to one subunit and peptidyl-tRNA to the other, and the subunits would alternate between tighter ("locked") and looser coupling. These intriguing speculations are not readily tested experimentally.

In eukaryotic systems the ribosome-subunit cycle seems to have much the same general features as in bacteria. Lysates contain single ribosomes and subunits, as well as polysomes; subunit exchange has been demonstrated⁶⁴; and initiation requires a special Met-tRNA (instead of fMet-tRNA)⁶⁵. Three protein initiation factors have been separated from rabbit reticulocyte ribosomes⁶⁶; and a dissociation factor, which acts on free but not on complexed ribosomes, has been extracted from ribosomal particles of a yeast⁶⁷ and of rabbit reticulocytes (unpublished results of N. Lubsen and myself). But there may be a significant difference in the cycle, since the ribosome pool does not equilibrate rapidly with added subunits or with freshly runoff ribosomes⁶⁸⁻⁷⁰.

Methodological Implications

The prolonged disagreement over the role of free ribosomes can be seen in retrospect to be due in part to the fact that the single 70S peak contains two kinds of ribosomes, whose difference in dissociability gave rise to experimental discrepancies. Another reason is the perhaps excessive influence of Occam's razor: though many initially simple biochemical processes are now known to involve complex regulatory mechanisms, a cycle that eliminated the inactive ribosome had wide appeal. Recently, however, the purification of DF and its identification with F₃ seem to have generated greater confidence in its function in ribosome dissociation⁷¹. But though purification is essential for analysing the mechanism of action of the factor in detail, the importance of this development should surely not be taken to imply that purification is also essential for establishing the existence of a factor or its significance in the cell.

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Construction of Maps from "Odd Bits of Information"

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Computer programs can produce very accurate maps from an apparently inadequate set of facts.

THE interesting communication¹ by Tobler and Wineberg illustrates well a rather general principle in historical geography, which is loosely described in the title of this article, and which forms the basis of a research programme currently being carried out in this laboratory. The idea behind the technique used here²⁻⁶ is to set up a suitable similarity (or dissimilarity) matrix $\Sigma=(\sigma_{ij})$ ($\Delta=(\delta_{ij})$) for the set of entities $\mathcal{E}_i, \mathcal{E}_j, \dots$ being studied, and believed to "lie naturally" in a Euclidean space of k dimensions, and then to carry out the following sequence of steps.

(i) Use Σ (Δ) together with a random initial $(k+1)$ -dimensional configuration to initiate a Shepard-Kruskal MD-SCAL analysis (see ref. 7 for Kruskal's first version of this) in $k+1$ dimensions. (The currently available programs should be modified to permit "colliding objects" to "pass through one another" during the iterated perturbations of the successive configurations; also isotropic rather than cartesian norming should be used at each iteration, and in the computer-plotted output each strongly linked pair of points (representing a pair of entities having a large σ_{ij} (a low δ_{ij})) should be shown with the link drawn on the plot.) (Of course, this remark about plotting only applies at this stage if $k=1$; it applies at stage (iii) if $k=2$.)

(ii) Project the final configuration on the first k "principal components", thus obtaining a k -dimensional configuration.

(iii) Use this k -dimensional configuration as the "prescribed" start of a k -dimensional MD-SCAL analysis, leading to a refined k -dimensional "map" in which, as far as possible, the distances d_{ij} (distance from point P_i representing entity $\mathcal{E}_i$ to point P_j representing entity $\mathcal{E}_j$) are monotonic decreasing functions of σ_{ij} (monotonic increasing functions of δ_{ij}).

(iv) Repeat the entire cycle with different "random starts".

Distortions and their Correction

Care must be taken to use the "primary" treatment of ties⁷ when, as sometimes happens, numerous ties occur among the entries in Σ (in Δ). If this is not done, gross distortions may result; for example, when $k=1$ and a large number of pairs of entities have equally large dissimilarities, a "horseshoe" shaped configuration may be obtained from data of linear origin⁴, but the use of the primary treatment of ties helps to correct for this. In the specific case of archaeological seriation⁶, an almost complete correction can be made by taking as similarity matrix not $\Sigma_0=AA'$ (where A is a zero-one incidence matrix for tombs against varieties), but the "circle product" $\Sigma_1=\Sigma_0 \circ \Sigma_0$, or, better still, an iterated circle product^{5,6,10}. The "local" rather than "global" ordering of similarities (or dissimilarities) is also very useful in such "sequencing" contexts (see recent modifications to MD-SCAL (unpublished) by Kruskal and his colleagues, and unpublished work by R. Gibson and by E. M. Wilkinson).

When $k=2$, we have the problem of Tobler and Wineberg, which they solve by an essentially similar technique. It therefore seems appropriate to report the success of two preliminary experiments carried out in Cambridge to validate our technique in the case $k=2$, as these also lend support to the work of Tobler and Wineberg.

In the first experiment, the "entities" were the eight parishes comprising the district of Otmoor in Oxfordshire, which is the object of a multidisciplinary study by Hiorns *et al.*, from which⁸ it was possible to obtain a standardized intermarriage rate for the eight parishes for a period extending roughly from AD 1600 to 1850, and this was taken as an index of similarity, σ_{ij} , for parishes $\mathcal{P}_i$ and $\mathcal{P}_j$. By carrying out the procedure I have described, a very accurate map of Otmoor was obtained from the computer⁶.

In the second experiment, the entities were the eighty-eight departments of France (Corsica and Paris excluded), and each one of the "odd bits of information" was simply whether or not one of the 3,828 pairs of departments shares a common boundary. Here the dissimilarity δ_{ij} for departments $\mathcal{D}_i$ and $\mathcal{D}_j$ was taken to be a generalized distance measure (suggested to me by Wilkinson): $\delta_{ij}=1+$ the smallest number of intermediate departments required to make up a connected chain from $\mathcal{D}_i$ to $\mathcal{D}_j$. Fig. 1 shows the resulting "map of France", and Fig. 2 shows the correct map. (The numbering of the departments is not official, but an alternative which approximately orders the departments first by longitude and then by latitude.) (Strictly speaking, the computer yields a "map" in which each department is represented by a point, but this system of linked points is converted to a "honeycomb" of cells by exploiting a natural duality.)

The next step in the programme will be to attempt to reconstruct a fifteenth century manor from the "abutments" in a

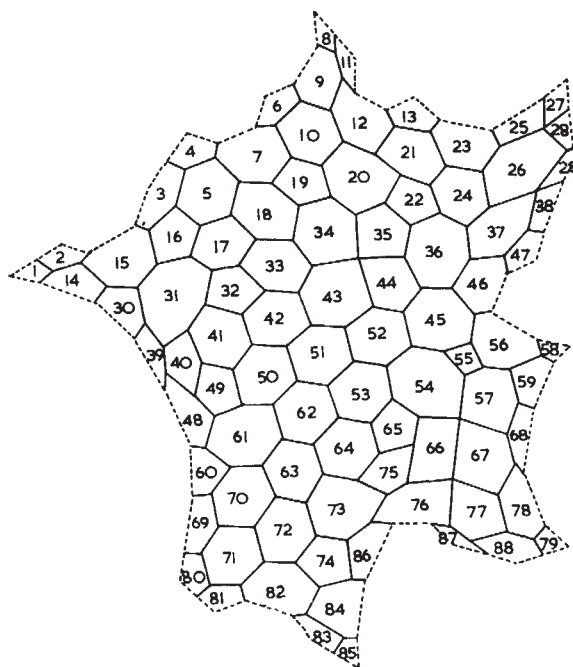


Fig. 1 France reconstructed from abutment data.



Fig. 2 France as it really is.

contemporary cartulary. This is being made possible by the cooperation of the Yorkshire Archaeological Society and the Department of Geography in the University of Leeds.

In conclusion it should perhaps be emphasized that: (i) MD-SCAL is not (or not primarily) a "clustering" programme; and (ii) it has numerous applications quite distinct from those discussed here. For example, ref. 9 shows the MD-SCAL and "clustering" techniques at work together on a single mapping problem in zoogeography.

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Hypsilophodon, the Cursorial Non-arboreal Dinosaur

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A reconsideration of the anatomy of *Hypsilophodon* from the Lower Cretaceous of England indicates that this ornithischian dinosaur, long considered to have been arboreal, was actually cursorial.

Hypsilophodon is a primitive bipedal ornithischian dinosaur (Fig. 1) and, like the other members of the sub-order Ornithopoda, was herbivorous. Several excellently preserved skeletons¹⁻³ of this small dinosaur, that reached a length of at least 7.5 feet, have been discovered in the Wealden Marls (Lower Cretaceous) of the Isle of Wight, England. The anatomical adaptations of *Hypsilophodon* have been discussed before^{2,4-10}. Only Heilmann⁹ has dissented from the opinion that *Hypsilophodon* was arboreal. *Hypsilophodon* is usually cited as the best example of an arboreal dinosaur, and many flesh reconstructions show it up trees^{4-8,10,11}. My re-examination of the evidence³ indicates that *Hypsilophodon* was not specifically adapted for an arboreal mode of life but was cursorial (Fig. 1).

Fore-arm Features

The humerus of *Hypsilophodon* is thought² to have had a wider range of brachial movement than in other ornithopods because it is longer than the scapula, its head is more medial in position and the delto-pectoral crest is more proximally placed (Fig. 2B). In the specimen (R5829)³ cited by Swinton², both scapulae are incomplete dorsally, and other specimens (R192, R196, R5830) demonstrate that the scapula is almost exactly the same length as the humerus (Figs. 2A and B). I

consider that the differences in position of the head of the humerus in various ornithopods are minimal and lack any real significance. The delto-pectoral crest (Fig. 2B) is proportionally in the same position as it is in *Dysalotosaurus*^{12,13}, *Iguanodon atherfieldensis*¹⁴, *Protoiguanodon*¹⁵ and *Protoceratops*¹⁶.

Abel⁴⁻⁶ thought that *Hypsilophodon* had a bowed radius bordering an enlarged fore-arm space and that, because of this, it differed from all other dinosaurs in the same way that the tree kangaroo *Dendrolagus* differs from the ground-living kangaroos. The radius of *Hypsilophodon*, however, is straight (Fig. 2C), and the fore-arm space is proportionally the same size or larger in *Thescelosaurus*¹⁷, *Dysalotosaurus*^{12,13}, *Camptosaurus browni*¹⁸, *Iguanodon atherfieldensis*¹⁴ and *Protoiguanodon*¹⁵ among the ornithopods.

There is no doubt that the manus (Fig. 2C) was capable of grasping, but its relatively small size (compare Figs. 1, 2C, 2E) would have limited its usefulness as a climbing aid. Examples of the manus are known from only a few of the lower ornithopods but those of *Thescelosaurus*¹⁷, *Protoiguanodon*¹⁵ and *Iguanodon atherfieldensis*¹⁴ were good grasping organs, as were those of prosauropods and theropods¹⁹. The grasping capabilities of the manus of *Hypsilophodon* resulted from the high degree of adaptation to bipedal locomotion.

Hind Limb and Tail

Hulke¹ and Abel⁴⁻⁶ were impressed by the length of the digits of the hind foot; Abel⁴⁻⁶ also thought that the phalanges had good flexural capabilities and that the unguals were very slender and pointed (Fig. 2F). In these features, however, the pes of *Hypsilophodon* (Figs. 1, 2E) does not differ from that of most other small to medium sized dinosaurs such as the other hypsilophodontids^{12,13,20}, *Thescelosaurus*¹⁷, psittacosaurids¹⁵, *Leptoceratops*¹⁶, prosauropods (Fig. 2D) and the smaller theropods¹⁹.

Abel⁴⁻⁶ thought that the hallux (the first digit of the pes) was opposable and this is the most important piece of evidence cited^{2,7,8,10,21} in support of the belief that *Hypsilophodon* was arboreal. In the reconstruction of the pes (Fig. 2F) given by Abel⁴⁻⁶, the hallux looks opposable because the ungual phalanx was restored the wrong way round; to agree with the pes (R196) as preserved in natural articulation³ (Fig. 2E) this ungual must be rotated through an angle of 180° around its longitudinal axis (pecked line in Fig. 2F). Swinton^{2,10} thought that the distal part of the first metatarsal diverged away from the second metatarsal and that the hallux was opposable to some extent. There is, however, no distal divergence^{1,3,4-6,8} (Figs. 2E and 2F), and the first metatarsal closely resembles that of *Parksosaurus*²⁰. In both genera the distal condyle is normal in form, so that during flexion the first digit moved parallel to or slightly away (medially) from the other digits as in all other dinosaurs. The supposed opposability of the hallux of *Hypsilophodon* is therefore based on misinterpretations of the material and, even if true (as in *Archaeopteryx*, *Anchisauripus* and some theropods), does not necessarily prove that the animal was arboreal²².

Swinton¹⁰ has recently reiterated his belief² that because the hind limbs were not adapted for rapid movement *Hypsilophodon* sought refuge from predators by going into the trees. Swinton² noted that the fourth trochanter of the femur is well down on the shaft, so that the caudi-femoral muscles would have hampered femoral movements to some extent. Admittedly the position of the fourth trochanter (Fig. 1) is low in comparison with that of most theropods, but it is more proximal in *Hypsilophodon than in any other post-Triassic ornithomimid³. Further, Swinton² noted that although the tibia is longer than the femur, the metatarsus is not elongated as in truly cursorial forms. To facilitate comparisons the hind limb ratios are given in Table 1. The distal part of the hind limb of *Hypsilophodon* (Fig. 1) is longer than the femur and because the hind limb is long relative to the trunk length this is not just a proportional change. Of all the ratios computed, those for *Hypsilophodon* (Table 1) are either the highest or among the highest for all post-Triassic ornithomimids^{3,23}. These values fall within the range given for the ungulates that Gregory²⁴ considered were cursorial, and all values are higher than in *Equus caballus**

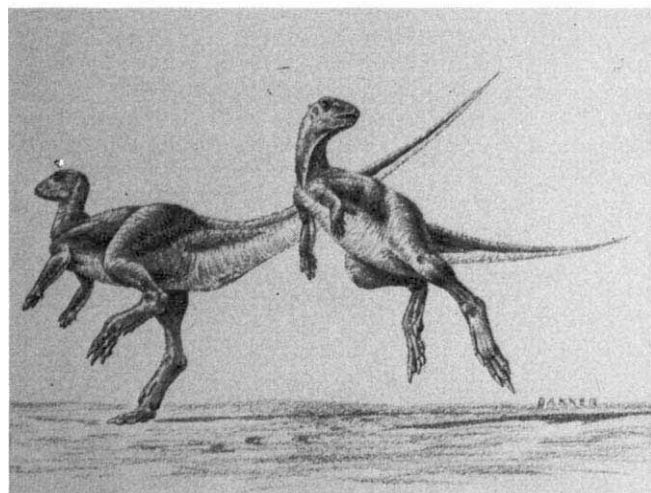
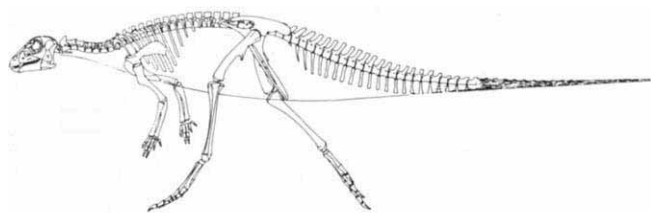


Fig. 1 *Hypsilophodon foxii*: skeletal (above) and flesh (below) reconstruction showing bodily proportions of an animal (R196) about 1.5 m or 4.5 feet long.

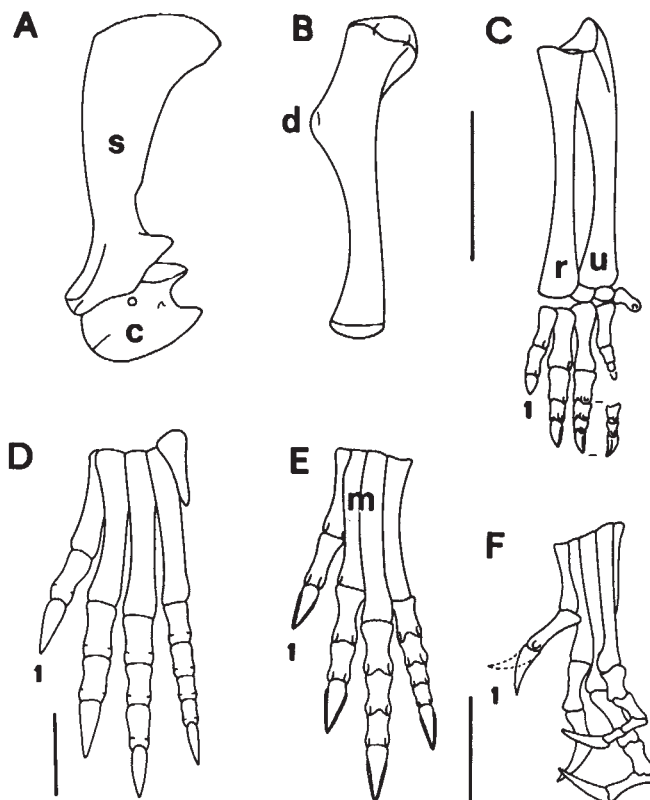


Fig. 2 A-C, E, F, *Hypsilophodon foxii*, R196, A, Left scapula and coracoid in lateral view; B, left humerus in lateral view; C, left fore-arm and manus in lateral view; E, left pes in dorsal view; F, right pes in ventro-medial view as restored by Abel⁴⁻⁶—pecked line indicates correct orientation of ungual phalanx I; D, *Anchisaurus colurus*, a slender footed prosauropod, right pes (Yale Peabody Museum No. 1883) in ventral view, note close resemblance to D. Abbreviations: c, coracoid; d, delto-pectoral crest; m, metatarsus; r, radius; u, ulna; 1, first digit. Vertical lines represent 5 cm; A, B and C drawn to the same scale as E and F.

(race horse). Most of the values are intermediate between those of *Coelophysis* and *Struthiomimus*, two coelurosaurian genera that are generally regarded as cursorial dinosaurs *par excellence*^{7,10,21}. *Hypsilophodon* was better adapted for fast running than any other post-Triassic ornithomimid described to date and it was cursorial.

Nopcsa²⁵ first questioned the function of the rigid posterior half of the tail which was ensheathed in ossified tendons (Fig. 1). This rigidity would have enhanced the use of the tail as a dynamic counterbalance when the animal was running. A similar tail is present in *Parksosaurus*²⁰, *Thescelosaurus*¹⁷, *Tenontosaurus*²⁶ and in the theropod *Deinonychus*¹⁹. With fast running as an effective means of escape it is unimportant

Table 1 Hind Limb Ratios of Dinosaurs and Cursorial Ungulates

	F	T	T+MT	MT	MT	HL
	in mm	F	F	F	T	TR
<i>Hypsilophodon</i> (R196)	151	1.18	1.73	0.56	0.47	1.53
<i>Parksosaurus</i> ²⁰	270	1.18	1.74	0.56	0.47	1.50
<i>Dysalotosaurus</i> ^{12,13}	366	1.10	1.69	0.59	0.54	1.44
<i>Camptosaurus nanus</i> ¹⁸	242	0.95	1.31	0.40	0.42	1.20
<i>Tenontosaurus</i> ²⁶	355	0.95	1.35	0.39	0.40	1.09
<i>Coelophysis bauri</i> ^{7,29}	209	1.07	1.67	0.60	0.56	1.20
<i>Struthiomimus altus</i> ³⁰	480	1.12	1.90	0.77	0.68	2.58
<i>Eohippus</i> sp. ²⁴	162	1.00	1.51	0.50	0.50	—
<i>Equus caballus</i> ²⁴	392	0.92	1.66	0.73	0.79	—
<i>Tragulus napu</i> ²⁴	94	1.09	1.75	0.66	0.60	—
<i>Gazella dorcas</i> (juv) ²⁴	140	1.25	2.20	0.81	0.75	—

Abbreviations: F, femur; HL, hind limb (=F+T+MT); MT, metatarsal III; T, tibia; TR, trunk (combined lengths of the centra of the dorsal vertebrae).

that the dermal armour²⁵ was light but, as discussed elsewhere³, it is not definitely established that *Hypsilophodon* was armoured.

Conclusion and Characterization

I conclude that *Hypsilophodon* shows no specific adaptations for an arboreal mode of life. The humerus did not have a wider range of brachial movement than in other ornithomimids, the radius is not uniquely bowed and the small size of the manus would have limited its usefulness as an aid in climbing. The form of the phalanges of the pes is very similar to those of most other small to medium sized dinosaurs and the hallux was not opposable. Because of the proximal position of the fourth trochanter and the elongate nature of the distal part of the hind limb, *Hypsilophodon* was more highly adapted for fast running than any other post-Triassic ornithomimid. Swinton¹⁰ notes that "arboreal", in its zoological sense, means adapted for living or moving about in trees. If *Hypsilophodon* is regarded as arboreal according to the second criteria then so must the following dinosaurs: all other hypsilophodontids including *Dysalotosaurus*, the iguanodontid *Thescelosaurus*, the pachycephalosaurid *Stegoceras*, the psittacosaurids, possibly *Leptoceratops*, the slender-footed prosauropods such as *Anchisaurus* (Fig. 2D) and all theropods (except mature individuals of the larger forms). It is more logical to regard *Hypsilophodon* as admirably well suited to a cursorial mode of locomotion. It shows no specific adaptations for an arboreal mode of life, and, if individuals did occasionally go up into the trees, then these excursions were no more frequent than in any other active dinosaur of comparable size.

Woodward²⁷ characterized the family Hypsilophodontidae as probably arboreal, while Romer²¹ postulated habits possibly somewhat arboreal in nature for ancestral ornithischians. With the transfer of the graviportal *Thescelosaurus* to the family Iguanodontidae, I characterize the family Hypsilophodontidae (which includes *Dysalotosaurus*) as follows²³: head small with short snout, large orbits, no canine teeth; cursorial with distal part of hind limb elongate. The cursorial hypsilophodontids seem to represent the basal and persistently primitive ornithischian stock^{3,22,23,28}.

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Specific Autostimulating Factor Released by Lymphocytes

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Early in the transformation of lymphocytes a factor has been found which is released and only stimulates DNA synthesis in other lymphocytes from the same or genetically similar donors as that from which the factor came.

RECENTLY a plethora of biologically active substances has been found in the culture medium of antigenically stimulated lymphocytes, some of which are capable of inducing mitosis in fresh lymphocytes¹⁻⁸. For example, the group which discovered that lymphocyte transformation occurred in mixed lymphocyte cultures⁹ also found a factor which stimulated allogeneic lymphocytes to synthesize DNA¹⁻⁵. The simplest interpretation—although not the only one¹⁰—is that this "mitogenic factor" contains HL-A antigens that stimulate lymphocytes lacking these antigens. Another "mitogenic factor" appears when lymphocytes from a Mantoux positive donor are exposed to antigens extracted from tubercle bacilli (for example, PPD), but this factor differs from the other in that it stimulates DNA synthesis in both allogeneic and autologous lymphocytes^{6,7}.

We now present evidence that during the early stages of transformation of lymphocytes in response to stimulation with concanavalin A (Con A)^{11,12} or in mixed cell cultures, a factor is released which differs from these mitogenic factors in that it affects only fresh lymphocytes from the same or genetically similar donors as those from which the factor was released. We will refer to it as the "autostimulating factor" (ASF). We have not looked for ASF in cultures in which sensitized lymphocytes are exposed to the antigens to which they are responsive, because the presence of soluble antigen in the medium would obscure the action of ASF.

In all our experiments venous blood samples were obtained from healthy donors; they were defibrinated and the red cells removed by sedimentation in 1% methyl cellulose. The granulocytes were removed by the addition of finely divided carbonyl iron and the remaining cells washed with culture medium TC 199. The lymphocytes in these cell preparations were transformed in culture either by the plant protein Con A¹¹ or by allogeneic lymphocytes, and the supernatants were assayed for ASF activity. The action of Con A on lymphocytes is to form complexes with glycoprotein on their surface, and further stimulation can be prevented by the presence of human serum^{11,12}, so avoiding the possibility of carry-over of Con A in the supernatant tested for ASF activity.

Table 1 shows a typical experiment using fresh lymphocytes from three unrelated donors *A*, *B* and *C* which were tested separately for stimulation of DNA synthesis after being cultured for 3 days in the presence of the filtrate from cultures of Con A-stimulated *A*, *B* or *C* lymphocytes. Such filtrates were capable of stimulating lymphocytes from the donor whose cells had been used in the original culture with Con A, but failed to stimulate lymphocytes from other donors. This experiment was repeated seven times with similar results. Separate experiments using autoradiography confirm that ASF stimulates DNA synthesis in autologous lymphocytes and produces morphological transformation.

ASF is not only found in cultures in which lymphocytes transform in response to stimulation by Con A, but it also

Table 1 ASF in Filtrates of Cultures from Con A-transformed Lymphocytes

Starting cultures producing ASF*	DNA synthesis induced by filtrates of "starting cultures" in lymphocytes from donors†		
	<i>A</i>	<i>B</i>	<i>C</i>
	c.p.m.	c.p.m.	c.p.m.
Cells <i>A</i>	47,846	741	564
	54,782	892	486
Cells <i>B</i>	788	48,197	819
	751	43,482	904
Cells <i>C</i>	809	519	28,417
	427	532	34,396

*Lymphocytes at a concentration of 1×10^6 ml.⁻¹ were suspended in serum-free culture medium TC 199 containing 1 mg ml.⁻¹ of three times crystallized Con A (obtained from Miles Laboratory) for 30 min at 37° C; the cells were then removed by gentle centrifugation (150g for 5 min), washed and re-suspended at a concentration of 1×10^6 cells ml.⁻¹ in fresh culture medium 199 containing 12.5% fresh autologous serum and methyl cellulose. Multiple sealed sterile tubes containing 3 ml. of these cell suspensions were incubated undisturbed at 37° C for 24 h and the culture was then filtered separately through sterile 'Millipore' filters (size 0.45 µm) to obtain the cell-free supernatant fluid.

† 2.5 ml. of the filtered culture medium to be tested for ASF was added to 0.5 ml. of TC 199 containing 3×10^6 freshly washed lymphocytes. These cultures were then maintained in sealed tubes at 37° C for varying times, after which DNA synthesis in the culture was determined by measuring the incorporation of radioactive thymidine. 15 µCi of ³H-thymidine (specific activity > 15 Ci mM⁻¹) was added and after 60 min at 37° C the cells were extracted by filtration onto a 'Millipore' filter. The cells on the filter were exposed to 10% ice-cold trichloroacetic acid washed with ethanol, and radioactivity was determined by liquid scintillation counting. Thymidine incorporation was expressed as c.p.m. In cultures in which there was no stimulation, c.p.m. were always less than 1,000 and values greater than 1,000 were regarded as a significant effect.

Table 2 ASF in Filtrates of Mixed Lymphocyte Cultures

Starting cultures producing ASF*	DNA synthesis induced by filtrates of "starting cultures" in lymphocytes from donors†			
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
	c.p.m.	c.p.m.	c.p.m.	c.p.m.
One-way mixed lymphocyte cultures:				
Cells <i>A</i> + cells <i>B</i> (mitomycin-treated) (1)	15,964	317	672	
	16,742	428	594	
Cells <i>B</i> + cells <i>A</i> (mitomycin-treated) (2)	586	12,398	594	586
	637	15,621	538	637
Cells <i>A</i> + cells <i>B</i> (after disruption by freezing and thawing) (3)	15,469	304	897	342
	16,742	412	904	518
Two-way mixed lymphocyte cultures:				
Cells <i>A</i> + cells <i>B</i> (no mitomycin) (4)	20,409	19,348	543	421
	24,392	17,642	592	507

* For all cultures, 1 million reacting lymphocytes were suspended in 1 ml. of culture medium TC 199 containing 12.5% of autologous serum. In cultures (1), (2) and (3) 0.5×10^6 stimulating allogeneic lymphocytes treated with 25 µg ml.⁻¹ of mitomycin C or exposed to repeated freezing and thawing were added. In these conditions the stimulating lymphocytes cannot themselves synthesize DNA. In cultures (4) neither population of lymphocytes was treated with mitomycin C and in this situation the lymphocytes stimulated each other. After incubation for 3 days in sealed tubes at 37° C, the supernatants from all the cultures were removed and filtered through 0.45 µm 'Millipore'.

† Thymidine incorporation was measured after the lymphocytes had been cultured for 3 days in the filtrates (see Table 1).

occurs in the filtered medium of mixed lymphocyte cultures. These mixed lymphocyte cultures were of two types: (1) the one-way culture, in which one of the populations of cells was prevented from synthesizing DNA by freezing and thawing or by prior treatment with mitomycin C (ref. 13); (2) in the two-way culture, the cells from the two donors stimulated each other.

The experiment illustrated in Table 2 was carried out with lymphocytes from four unrelated donors (*A*, *B*, *C* and *D*). The results show that the filtrate removed from one-way cultures after 3 days could stimulate only fresh lymphocytes from the same donor as the lymphocytes that had responded in the original culture. Fresh lymphocytes from the donor of the "stimulating lymphocytes" (that is, the mitomycin treated or frozen cells) were not stimulated by the cell-free filtrate. But when the filtrate from the two-way mixed lymphocyte culture (that is, from *A* and *B*) was incubated with fresh lymphocytes from either donor *A* or *B*, both were stimulated, but lymphocytes from all other donors were unaffected. This experiment was repeated using the cultures of seven different lymphocyte donors whose cells had been stimulated by allogeneic lymphocytes. In every case, the filtrate only stimulated fresh cells if they were from the same donor as the stimulated cells.

We have found that the lymphocytes from patients with acute leukaemia are transformed in culture by the presence of autologous leukaemia cells, presumably because leukaemia cells possess tumour associated antigens¹⁴. The supernatants from such cultures also exhibit typical ASF activity.

A significant feature of the experiments reported in Tables 1 and 2 is the failure of the culture medium to stimulate any allogeneic cell. Our inability to find the type of activity associated with the mitogenic factor of Kasakura³⁻⁵ which acts against allogeneic cells can be ascribed to the timing of the cultures. In the experiments described by Kasakura, mitogenic activity was determined in the medium from mixed lymphocyte cultures by culturing with fresh lymphocytes for 5-7 days and it was found to have a similar time course and specificity to stimulation of lymphocytes by HL-A antigens¹⁵. In our experiments (Tables 1 and 2) the mitogenic activity of media on fresh lymphocytes was studied after 3 days. ASF produces maximum DNA synthesis in fresh autologous cells after

approximately 3 days (Fig. 1), regardless of its mode of production (that is, whether produced in either cultures of cells transformed by Con A (curve I) or in mixed cell cultures (curve II)). If the supernatant (in this case the same media as used for curve II) is tested for its action on allogeneic cells (curve III), however, there is no mitogenic activity at 3 days but marked stimulation occurs at 6 days at which time there is still ASF activity.

It is conceivable that the mitogen factor described by Wolstencroft and Dumonde⁷ studied in 6 day cultures, which is equally active on all lymphocytes whether allogeneic or autologous, may be a mixture of ASF and the Kasakura factor as we found in 6 day cultures.

In the experiments described so far, the filtrate taken from cultures of stimulated lymphocytes induced DNA synthesis only in autologous lymphocytes and not in those from unrelated donors. The possibility remains, however, that ASF may stimulate lymphocytes from donors who are closely related genetically. That this is the case is shown by two family studies (Table 3) in which ASF from lymphocytes of one sibling stimulated lymphocytes from another who shared common HL-A antigens. These studies are clearly not sufficient to define factors determining the specificity of ASF and investigations using the lymphocytes from other groups of siblings are in progress.

In all these experiments, the presence of ASF was detected by suspending fresh lymphocytes in serum containing medium in which cells had already been cultured for either 24 h after Con A-stimulation, or 3 days with allogeneic lymphocytes. If to this medium, which had been conditioned by the growth of cells in it, was added medium containing fresh serum, no ASF activity would be detectable (Table 4). The simplest interpretation of these findings is that serum contains a factor (or factors) which interferes with the action of ASF and that this

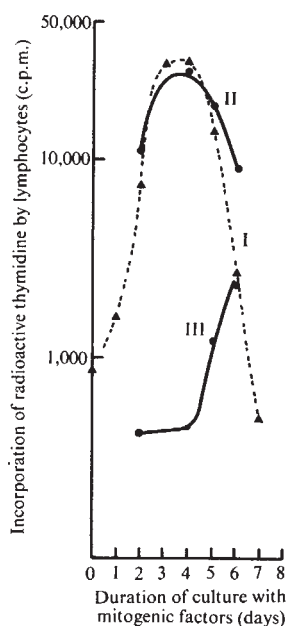


Fig. 1 Kinetics of stimulation of lymphocytes by ASF or the mitogenic factor which acts against allogeneic cells (Kasakura-like).

ASF: thymidine incorporation of fresh lymphocytes (A) cultured for different periods in filtrates from cultures from: (I) lymphocytes (A) stimulated with Con A and cultured for 24 h; (II) lymphocytes (A) cultured for 3 days together with mitomycin-treated allogeneic lymphocytes.

Allogeneic mitogenic factor: thymidine incorporation of fresh lymphocytes (B) cultured for different periods in filtrates from cultures of: (III) lymphocytes (A) cultured for 3 days together with mitomycin-treated allogeneic lymphocytes (the same filtrate used for curve II).

Table 3 Effect of ASF on Lymphocytes of Siblings* (B and M are Normal Donors unrelated to the Two Families Studied)

Cells in starting culture	ASF from starting cultures on fresh lymphocytes from †			
	Sib. L	Sib. A	B	R
Sib. L + B (mitomycin-treated)	15,649	12,842	517	489
	17,421	18,728	419	503
Sib. A + B (mitomycin-treated)	8,642	14,681	523	691
	10,439	13,937	487	632
Identical twins	Twin R	Twin T	B	M
Twin R + B (mitomycin-treated)	25,369	10,369	512	678
	27,491	12,491	618	743
Twin T + B (mitomycin-treated)	12,412	19,368	674	415
	14,362	24,839	580	473

* HLA antigens were Sib. L, 2.3.12; Sib. A, 2.3.8; twin R, 2, Ba*, LND, BB; twin T, 2, Ba*, LND, BB; donor B, 2.3.5. Stimulation in mixed lymphocyte reaction (c.p.m.):

Sib. A + Sib. L (mitomycin-treated)	3,967
	4,572
Sib. L + Sib. A (mitomycin-treated)	154
	279
Sib. A + M (mitomycin-treated)	10,320
	11,613
Sib. L + M (mitomycin-treated)	11,726
	11,002
Twin R + Twin T (mitomycin-treated)	460
	527
Twin T + Twin R (mitomycin-treated)	370
	416
Twin R + M (mitomycin-treated)	22,061
	20,638
Twin T + B (mitomycin-treated)	19,714
	21,181

† DNA synthesis induced by filtrates of "starting cultures" on fresh lymphocytes from donors L, A, B and R (see Table 1), cultured for 3 days.

Table 4 Dose Response Relationship for ASF Activity

Composition of culture medium: Volume ASF* from cells (A) (ml.)	Volume medium 199 † (ml.)	ASF activity ‡ (expressed as c.p.m.) on	
		Lymphocytes (A)	Lymphocytes (B)
2.5	0.0	58,141	845
		62,112	816
1.25	1.25	21,386	248
		19,612	297
0.75	1.75	14,209	415
		16,841	
0.375	2.125	3,414	397
		2,947	
0.18	2.32	1,001	304
0.09	2.41	485	383
1.5	1.0, conditioned serum§	10,348	
		8,635	
1.5	1.0, fresh autologous serum	438	
		296	
1.5	1.0, fresh allogeneic serum	547	
		609	

* Lymphocytes (A) at a concentration of 1×10^6 ml.⁻¹ were stimulated by Con A (1 mg ml.⁻¹) in medium 199 for 20 min at 37° C then washed in medium 199 containing 12.5% serum which binds and removes unutilized Con A. The lymphocytes were resuspended in serum-free medium 199 at a concentration of 1×10^6 ml.⁻¹ in 3 ml. aliquots and incubated at 37° C for 24 h. The cultures were then filtered to obtain cell-free supernatant fluid containing ASF.

† The volume of medium 199 made the total volume of all cultures for ASF assay 3 ml.

‡ 3×10^6 lymphocytes from donors (A) and (B) were incubated with the culture medium for 3 days at 37° C and incorporation of radioactive thymidine was then measured.

§ Medium 199 containing 20% serum. The conditioned serum was a sample of ASF from cells (B) and thus not active on cells (A) but containing 20% serum that had been conditioned by the 24 h culture period required to produce the ASF (B).

inhibitory factor is destroyed by the lymphocytes as they are undergoing transformation in the starting culture medium in which the ASF is being produced. There are other examples in which the medium derived from a growing culture is able to support the growth of other cells better than fresh culture medium^{16,17}.

Because of the antagonistic action of fresh serum, a dilution experiment to determine a dose response relationship for ASF requires that the diluent contains no serum. In our experiment shown in Table 4, ASF was produced in a serum-free culture of Con A-stimulated lymphocytes and then tested in various dilutions for its capacity to stimulate fresh lymphocytes. The degree of stimulation produced was proportional to the volume of the medium containing ASF and the ASF activity remained specific for the autologous lymphocytes.

The nature and the biological role of ASF and its unique type of specificity will remain unclear until information concerning its chemical constitution has been obtained and there has been more detailed study of the precise degree of specificity which it exhibits. ASF may have a recruitment function, in the transformation of lymphocytes in mixed cell cultures in which maximum DNA synthesis occurs 6 days after antigenic stimulation; but ASF is produced much sooner, commencing within 1 day and reaching a peak at after 2.5 days. Possibly, the sequence of events is that a relatively small number of cells is transformed between 2–4 days as a result of specific antigenic stimulation during which ASF is released, and that this is followed by the transformation of a large number of cells by the ASF.

Thus, the mixed lymphocyte reaction may be considered to be a cascade phenomenon, in which a few lymphocytes transform quite early thereby initiating a chain reaction with a peak response after 6 to 7 days.

The experiments in which lymphocytes from one donor were stimulated with Con A (Table 1) leave no doubt that the lymphocyte population which is capable of responding to the ASF is the same as that which releases this material into the culture medium. We therefore feel justified in referring to this substance as a factor which specifically stimulates autologous cells.

ASF differs from all the mitogenic factors previously

described in the type of specificity it exhibits; that is, a particular population of cells produces a factor which is only capable of stimulating lymphocytes from the same donor or from one closely related genetically. Clearly such a substance cannot be an antigen, but its ability to distinguish lymphocytes from unrelated donors implies that the specificity exhibited must be immunological in nature.

Because antigen recognition sites may not exhibit specificity only for the antigen to which they respond, but also combine specifically to the surface of the cell by which they are produced, we are exploring the idea that ASF may be related to the antigen recognition sites on the lymphocytes.

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Enzyme Regulation, Lysine Pathways and Cell Wall Structures as Indicators of Major Lines of Evolution in Fungi

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The distribution of the two known pathways of lysine biosynthesis can be correlated with allosteric controls of enzymes and dichotomy of cellulose and chitin in fungi.

INFORMATION about the evolutionary relationships between species has been obtained by comparing their modes of enzyme regulation^{1–9}, the primary structures of their proteins¹⁰ and the patterns of their biochemical pathways^{8,11}. In fungi, information is also available on cell wall structure¹² but it has been

used little in evolutionary studies. In our studies of the phylogenetic origins of the fungi, we have attempted to combine all four approaches, but because data on the primary structure of the fungal enzymes are not yet available, we have had to rely heavily on a comparison of the regulatory properties of the enzymes. We compared the regulatory properties of glutamic dehydrogenase, an enzyme crucial to nitrogen and carbon metabolism, and other regulatory enzymes of different species of fungi, placing emphasis on the lower fungi because of the uncertainties that exist about their phylogenetic origins¹³.

Glutamic Dehydrogenase Variants

More than forty species of lower fungi, Myxomycetes and Phycomycetes, were found to possess only an NAD-linked

glutamic dehydrogenase. The higher fungi, Deuteromycetes, Ascomycetes and Basidiomycetes, seem to produce two distinct forms of the enzyme, one NAD-linked, the other NADP-linked (Table 1). The unique distribution of these two coenzyme-specific forms provided an opportunity to determine what mechanisms of enzyme regulation of the glutamic dehydrogenases are operative in these fungi.

The NAD-linked glutamic dehydrogenases of the Phycomycetes can be divided into three classes on the basis of their regulatory properties (Table 2). In contrast, nearly all the Chytridiales and Mucorales¹⁴ possess unregulated forms of glutamic dehydrogenase, described as type I enzyme in Table 2. Type II was found in members of the Blastocladales, a largely aquatic group, and in *Absidia*, a genus of the Mucorales. Type II enzymes have a complex multivalent mode of regulation. Divalent metal ions such as Ca^{2+} and Mn^{2+} activate the reductive amination reaction but inhibit the oxidative deamination reaction. In nonequilibrium conditions, therefore, the control is unidirectional^{15,16}. Adenylates modulate the activity such that in conditions of high "energy charge"¹⁷, glutamate breakdown is inhibited but its synthesis is enhanced^{15,16}. The influence of divalent metal ions on the type II enzyme may be connected with the freshwater habit of these fungi: although Ca^{2+} and Mn^{2+} are present in relatively small concentrations

in freshwater, they seem to induce motile zoospores to enter a germinating phase of development¹⁸. In soil, Ca^{2+} and Mg^{2+} concentrations are generally large, and it was surprising, therefore, to find that *Absidia*, the Mucorales genus which seems to be well adapted to a terrestrial environment, disseminating nonmotile, "dry" spores, possesses type II enzyme. To begin with, this raised the interesting possibility that convergent evolution of an enzyme control mechanism may have occurred in these fungi. The distribution of type II enzyme among the Mucorales is unknown, however, because our survey is incomplete (Table 1).

Type III enzymes have proved to be the most informative. Found so far only in the Oomycetes and Hypochytridiomycetes, they are defined as those that use NAD^+ as a substrate in catalysis, only interacting with NADP^+ when it functions as an allosteric modulator¹⁹. GTP and ATP inhibit type II enzymes²⁰ (Table 2) but activate type III enzymes with the exception of one "subclass". AMP, an activator of type II enzyme, inhibits the type III form. Other activators of the type III enzyme are listed in Table 2. Both type II and type III are strongly inhibited by citrate^{18,19}. The "subclass" with the type III enzyme that is not activated by GTP and ATP is *Rhizidiomyces* of the Hypochytridiomycetes. These are organisms with an uncertain taxonomic position. It has been

Table 1 Distribution of Glutamic Dehydrogenases in Fungi

Organism	Order	Class	Coenzyme NAD-linked	Specificity NADP-linked
<i>Dictyostelium discoideum</i>	Acrasiales	Myxomycetes	+	—
<i>Polysphondylium violaceum</i>	Acrasiales	Myxomycetes	+	—
<i>Entophlyctis</i> sp. (P)	Chytridiales	Chytridiomycetes	+	—
<i>Rhizophlyctis rosea</i> (P)	Chytridiales	Chytridiomycetes	+	—
<i>Rhizophydium sphaerocarpum</i> (P)	Chytridiales	Chytridiomycetes	+	—
<i>Allomyces arbuscula</i> (P)	Blastocladales	Chytridiomycetes	+	—
<i>Blastocladiella emersonii</i> (P)	Blastocladales	Chytridiomycetes	+	—
<i>Rhizidiomyces apophysatus</i> (P)	Hypochytriales	Hypochytridiomycetes	+	—
<i>Hypochytrium catenoides</i>	Hypochytriales	Hypochytridiomycetes	+	—
<i>Achlya</i> sp. (1969) (P)	Saprolegniales	Oomycetes	+	—
<i>Achlya bisexualis</i> (male)	Saprolegniales	Oomycetes	+	—
<i>Achlya bisexualis</i> (female)	Saprolegniales	Oomycetes	+	—
<i>Achlya americana</i>	Saprolegniales	Oomycetes	+	—
<i>Isoachlya itoana</i> (P)	Saprolegniales	Oomycetes	+	—
<i>Saprolegnia parasitica</i>	Saprolegniales	Oomycetes	+	—
<i>Saprolegnia turfosa</i> (P)	Saprolegniales	Oomycetes	+	—
<i>Saprolegnia ferax</i>	Saprolegniales	Oomycetes	+	—
<i>Thraustotheca clavata</i>	Saprolegniales	Oomycetes	+	—
<i>Aphanomyces euteiches</i>	Leptomitales	Oomycetes	+	—
<i>Sapromyces androgynus</i> (P)	Leptomitales	Oomycetes	+	—
<i>Apodachlya</i> sp. (Strain 47-17) (P)	Leptomitales	Oomycetes	+	—
<i>Leptomitus</i> sp. (Strain 59-65) (P)	Leptomitales	Oomycetes	+	—
<i>Pythium debaryanum</i>	Peronosporales	Oomycetes	+	—
<i>Pythium undulatum</i>	Peronosporales	Oomycetes	+	—
<i>Pythium catenulatum</i>	Peronosporales	Oomycetes	+	—
<i>Pythium splendens</i>	Peronosporales	Oomycetes	+	—
<i>Phytophthora cinnamomi</i>	Peronosporales	Oomycetes	+	—
<i>Phytophthora palmivora</i>	Peronosporales	Oomycetes	+	—
<i>Absidia glauca</i>	Mucorales	Zygomycetes	+	—
<i>Cunninghamella blakesleeana</i>	Mucorales	Zygomycetes	+	—
<i>Mucor hiemalis</i>	Mucorales	Zygomycetes	+	—
<i>Rhizopus stolonifer</i>	Mucorales	Zygomycetes	+	—
<i>Zygorhynchus mollieri</i>	Mucorales	Zygomycetes	+	—
<i>Phycomyces blakesleeana</i>	Mucorales	Zygomycetes	+	—
* <i>Piricularia oryzae</i>	Moniliales	Deuteromycetes	+	+
* <i>Fusarium oxysporum</i>	Moniliales	Deuteromycetes	+	+
* <i>Candida utilis</i>	Moniliales	Deuteromycetes	+	+
<i>Hypomyces rosellus</i>	Hypocreales	Ascomycetes	+	+
* <i>Saccharomyces cerevisiae</i>	Endomycetales	Ascomycetes	+	+
* <i>Hansenula subpelliculosa</i>	Endomycetales	Ascomycetes	+	+
<i>Sordaria fimicola</i> (+ and -)	Sphaeriales	Ascomycetes	+	+
* <i>Neurospora crassa</i>	Sphaeriales	Ascomycetes	+	+
* <i>Coprinus lagopus</i>	Agaricales	Basidiomycetes	+	+
* <i>Schizophyllum commune</i>	Agaricales	Basidiomycetes	+	+

* Data taken from Casselton, P. J. (ref. 37).

Organisms marked (P) were obtained from the personal collection of Dr J. S. Lovett, of Purdue University, Drs E. S. Beneke and A. Rogers, of Michigan State University, Dr M. Fuller, of the University of Georgia, and Dr R. Emerson, of the University of California (Berkeley). Specific details can be supplied on request. The other fungi were obtained from commercial sources.

argued that they are Oomycetes because of their zoospore flagella structure and cell wall composition²¹. But their cell morphology and habitat suggest that it is more appropriate to place them among the Chytridiomycetes²². The uncertainty of their definition is reflected in the manner in which their glutamic dehydrogenase is controlled.

Glutamic dehydrogenase from the Hypochytridiomycetes is controlled in a manner intermediate between those of types II and III. For example, AMP activates *Rhizidiomyces* glutamic dehydrogenase whereas ATP and GTP inhibit it. This pattern of regulation by purine nucleotides has been observed in *Blastocladiella*²⁰ and *Allomyces* (unpublished data of F. Yen and H. B. L.) which possess the type II enzyme. Other activators (NADP⁺, P-enolpyruvate and short chain acyl CoA derivatives) and inhibitors (citrate and long chain acyl CoA derivatives) of type III enzymes present in the Oomycetes (Saprolegniales, Peronosporales and Leptomitales) function in the same way as in the Hypochytridiomycetes (Table 2)²³.

The intermediate mode of enzyme control of the Hypochytridiomycetes raises the question of a possible evolutionary link between the Chytridiales and the Oomycetes. Are they genetic hybrids or are they forerunners of the latter group? These enzyme studies may indicate that they are forerunners in support of earlier conclusions based on cell structures²⁴.

When Oomycetes and Hypochytridiomycetes are grown in the presence of glucose or sucrose and limited amounts of amino-acids, their glutamic dehydrogenase production is repressed¹⁹. The enzyme production in the Chytridiales, Blastocladiiales and Mucorales, on the other hand, is not significantly affected in these conditions. Glutamate and a variety of amino-acids that are precursors of glutamate can serve as inducers of the repressed enzyme in the Oomycetes and Hypochytridiomycetes. This change has been shown to be due to repression at the levels of transcription and translation (Smaluck, Lees and LeJohn, manuscript in preparation).

Lactic Dehydrogenase

Because data from single enzyme systems may produce spurious evolutionary correlations, we extended our studies to include one enzyme that is involved in anaerobic metabolism and one that operates in aerobic pathways. D(-) lactic dehydrogenases found in the Phycomycetes fall into two distinct categories. One (class I), present only in the Hypochytridiomycetes and Oomycetes, is powerfully and allosterically inhibited by GTP²⁵. It has been shown that GTP specifically regulates the levels of NADH and lactate but not pyruvate and NAD⁺. ATP also inhibits the enzyme but its effect can be explained simply, without recourse to allostery, for it acts as a competitive inhibitor at the pyridine nucleotide binding sites. The second category (class II) of D(-) lactic dehydrogenase is found in the Chytridiomycetes and Zygomycetes. This class has not been shown to elicit any form of control except the typical competitive inhibition by ATP. Class I D(-) lactic dehydrogenases of glucose and sucrose grown cells, in contrast to those of pyruvate and amino-acid grown cells, are considerably repressed.

Isocitric Dehydrogenases

The Phycomycetes contain an NADP-linked isocitric dehydrogenase which does not have any significant allosteric properties. The only observable difference between the enzyme in the Chytridiomycetes and that in the Oomycetes is that in the first it catalyses a freely reversible reaction whereas, in the second, the reaction cannot be reversed in any of our assay conditions (unpublished result of H. B. L.).

NAD-linked isocitric dehydrogenases are also present in the Chytridiomycetes and Zygomycetes and are usually allosteric but they are absent from the Oomycetes and Hypochytridiomycetes. AMP activates the enzyme. The enzyme is activated by citrate only in the Blastocladiiales²⁶ of the Chytridiomycetes. Citrate inhibits all the other NAD-linked isocitric dehydro-

Table 2 Different Types of NAD-linked Glutamic Dehydrogenase found in Phycomycetes

Possible order of evolution	Enzyme type	Activators	Inhibitors	Glucose repression of synthesis
Chytridiales and most Mucorales	Type I	None	None	Negative
Blastocladiiales	Type II	Ca ²⁺ , Mn ²⁺ , AMP	Citrate, ATP, FDP, EDTA, GTP	Negative
Few Mucorales	Type II	Ca ²⁺ , Mn ²⁺ , AMP	Citrate, ATP, FDP, EDTA, GTP	Negative
Hypochytridiales	Type III (intermediate?)	NADP ⁺ , NADPH, AMP, acetyl CoA and short chain CoA derivatives, P-enolpyruvate	ATP, citrate, GTP, long chain acyl CoA derivatives, Ca ²⁺ , Mn ²⁺	Positive
[Saprolegniales Leptomitales Peronosporales]	Type III	NADP ⁺ , NADPH, GTP, ATP, acetyl CoA and short chain acyl CoA derivatives, P-enolpyruvate	AMP, citrate, long chain acyl CoA derivatives, Ca ²⁺ , Mn ²⁺	Positive

A possible evolutionary pattern for Chytridiales, Blastocladiiales, Hypochytridiales, Saprolegniales, Leptomitales, Peronosporales, and Mucorales based on enzyme control mechanisms. Glutamic dehydrogenases of the Oomycetes and Chytridiomycetes marked (±) were purified as described before^{15,19,20}. Homogeneity of the enzymes from *Blastocladiella*, *Allomyces*, *Achlya* sp. and *Pythium debaryanum* have been ascertained by analysis on polyacrylamide gels. The enzyme preparations used for the Chytridiales, Acrasiales and Mucorales were obtained by ammonium sulphate (45–60% saturation) fractionation of cell free extracts and filtration of the solubilized protein through 'Sephadex G-25' columns (1.5 × 30 cm). Partially purified enzyme from all other members of the Oomycetes was obtained after removal of nucleic acids and some proteins by precipitation with protamine sulphate (0.66 mg/ml.) and centrifugation; fractionation of the supernatant solution with ammonium sulphate (50–65% saturation) and filtration of the dissolved proteins through 'Sephadex G-25' column. In some cases, further purification was achieved by chromatographing on DEAE-cellulose (2 × 20 cm) and eluting with 0.15 M KCl in buffer. The enzyme was recovered by ammonium sulphate fractionation (50–70% saturation). A standard buffer of composition, 0.05 M Tris, 0.01 M phosphate and 0.001 M EDTA adjusted to pH 7.3 with acetic acid was used throughout. Cell extracts were processed in volumes varying from 5 ml. to 25 ml. and the protein content was between 5 and 10 mg/ml. Assay methods used are described in refs. 15, 19 and 20. D(-) Lactate dehydrogenases have been purified from *P. debaryanum*, *P. splendens*, and *Achlya* sp. only. Complete details have been reported²⁵. In every other case, protein precipitating between 50 and 65% saturation with ammonium sulphate was used as the source of the enzyme. NADP-linked isocitric dehydrogenases from *Entophlyctis* sp., *Achlya* sp., *Thraustotheca* and *P. debaryanum* were partially purified by DEAE-cellulose chromatography, ammonium sulphate (45–55% saturation) fractionation and filtration through 'Sephadex G-200' (2.5 × 100 cm). The enzymes from *Blastocladiella* and *Rhizopus* were partially purified by similar procedures plus acetone fractionation (40%) of the cell free extracts as an initial step. Enzymes were kept in 20% glycerol-phosphate buffer for stability. The method for purification of *Blastocladiella* and *Allomyces* NAD-linked isocitric dehydrogenases has been reported²⁶.

genases tested in the Phycmycetes (Table 3). In this respect, the Blastocladales and Sphaeriales (Ascomycetes) are similar.

Lysine Pathways and Cell Wall Structure

Vogel²⁷ has shown that there are two pathways of lysine biosynthesis. Both the α,ϵ -diaminopimelic acid (DAP) pathway and the α -amino adipic acid (AAA) pathway operate in the fungi²⁷. Although bacteria, a few fungi and all the higher plants examined make lysine exclusively by the DAP pathway, the AAA lysine pathway seems to be largely confined to fungi and euglenids²⁷.

We have observed that all fungi using the DAP biosynthetic pathway (1) contain type III glutamic dehydrogenase, (2) have poorly defined cellulose cell walls²⁸, (3) contain hydroxyproline in place of proline as a constituent amino-acid of their walls²⁹, (4) have a GTP modulated D(-) lactic dehydrogenase (class I) and (5) have glutamic and D(-) lactic dehydrogenases that are sensitive to catabolite repression by carbohydrates. Those fungi such as the Ascomycetes, Deuteromycetes and Basidiomycetes that synthesize lysine by the AAA pathway have none of these properties (Table 4).

Cellulose and chitin are distributed in fungi in much the same way as the DAP and AAA lysine biosynthetic pathways. For example, three species, *Rhizidiomyces apophysatus*²⁴ (a member of the Hypochytridiomycetes), *Apodachlya* sp. (an Oomycete³²) and *Ceratocystis ulmi*³³ (an Ascomycete), containing both cellulose and chitin in their cell walls, also lack a specific control by uridylates and UDP-amino sugar derivatives. These compounds activate the glutamic dehydrogenase from Oomycetes which do not contain chitin³⁴ (H. B. L., manuscript in preparation). It seems, therefore, that the control mechanisms of chitin (amino sugar) biosynthesis and glutamate and glutamine biosynthesis are connected.

The chitin-cellulose: AAA-DAP correlation in fungi seems to hold true for all living organisms. Vogel²⁷ has shown that *Euglena* is an intriguing exception in the evolutionary pattern proposed for the two lysine paths. It is interesting that *Euglena* does not contain cellulose and the AAA pathway is operative. *Chlorella pyrenoidosa* which contains 15.6% α -cellulose and 31% hemicellulose in its wall³⁵ utilizes the DAP path. Both are green algae. Higher plants and blue-green algae possess cellulosic cell walls³⁵ and they too rely on the DAP pathway for lysine synthesis.

Phylogenetic Implications

It is possible that the correlations we have observed have roots in ancestral selection processes. It seems likely, as Vogel suggests, that the DAP path preceded the AAA path in time, and, similarly, that cellulose biosynthesis preceded chitin biosynthesis. If this is true, it can be assumed that there was some selective advantage inherent in the development of an entirely new and elaborate lysine biosynthetic pathway (AAA) early in fungal evolution. If DAP-lysine metabolism interferes in some way with chitin biosynthesis, and chitin was necessary, for example, to combat cellulolytic action of other soil microorganisms and/or to provide the proper polymeric cell wall structure for altered morphogenesis, then there could have been cause for the DAP path to be eliminated. Chitin is known to be far more resistant to microbial decomposition than cellulose. This may be one of several reasons why the AAA biosynthetic path is found largely in fungi and seldom in any other living organisms. On the basis of enzyme controls, cell wall structure and lysine pathway, the Hypochytridiomycetes are probably forerunners of the Chytridiales and the Oomycetes, not an intermediate.

A phylogenetic scheme based on an analysis of enzyme

Table 3 Distribution and Regulatory Properties of Isocitric and D(-) Lactic Dehydrogenases in the Phycmycetes

Order	Isocitric dehydrogenases	D(-) Lactic dehydrogenases
Chytridiales	NAD-linked: AMP activation; ATP, citrate inhibition	ATP inhibition (competitive)
Blastocladales	NADP-linked: no significant regulation evident; reversible	ATP inhibition (competitive)
Hypochytridiales, Saprolegniales, Leptomitales	NAD-linked: AMP and citrate activation; ATP inhibition	GTP inhibition (allosteric)
Peronosporales	NADP-linked: ATP, citrate as inhibitors; reversible	ATP inhibition (competitive)
Mucorales	Only NADP-linked: irreversible	
	NAD-linked: AMP activation; ATP, citrate inhibition	ATP inhibition (competitive)
	NADP-linked: reversible	

Table 4 Correlations between Pathways of Lysine Biosynthesis, Cell Wall Structure, Glucose Effect, Multivalent Control of Glutamic Dehydrogenases (NAD-linked) and D(-) Lactic Dehydrogenases of Fungi

Order	Lysine path	Cellulose in wall	Chitin in wall	Type of glutamic dehydrogenase	D(-) LDH class	Sensitivity to glucose effect
Acrasiales	DAP*	+	-	Type III	I	?
Chytridiales	AAA	-	+	Type I	II	Negative
Blastocladales	AAA	-	+	Type II	II	Negative
Hypochytridiales	DAP	+	+	Type III (intermediates)	I	Positive
Saprolegniales	DAP	+	-	Type III	I	Positive
Leptomitales	DAP	+	-	Type III	I	Positive
Lagenidiales	DAP	+	-	Type III	I	Positive
Peronosporales	DAP	+	-	Type III	I	Positive
Mucorales	AAA	-	+	Types I and II	II	Negative
Higher fungi						
Endomycetales	AAA	-	+		II	Negative
Plectascales	AAA	-	+			Not known
Sphaeriales	AAA	-	+			Negative
Ustilaginales	AAA	-	+			Not known
Agaricales	AAA	-	+			Not known

* Predicted from evolutionary pattern. +, Present; -, absent; no entry indicates results indefinite.

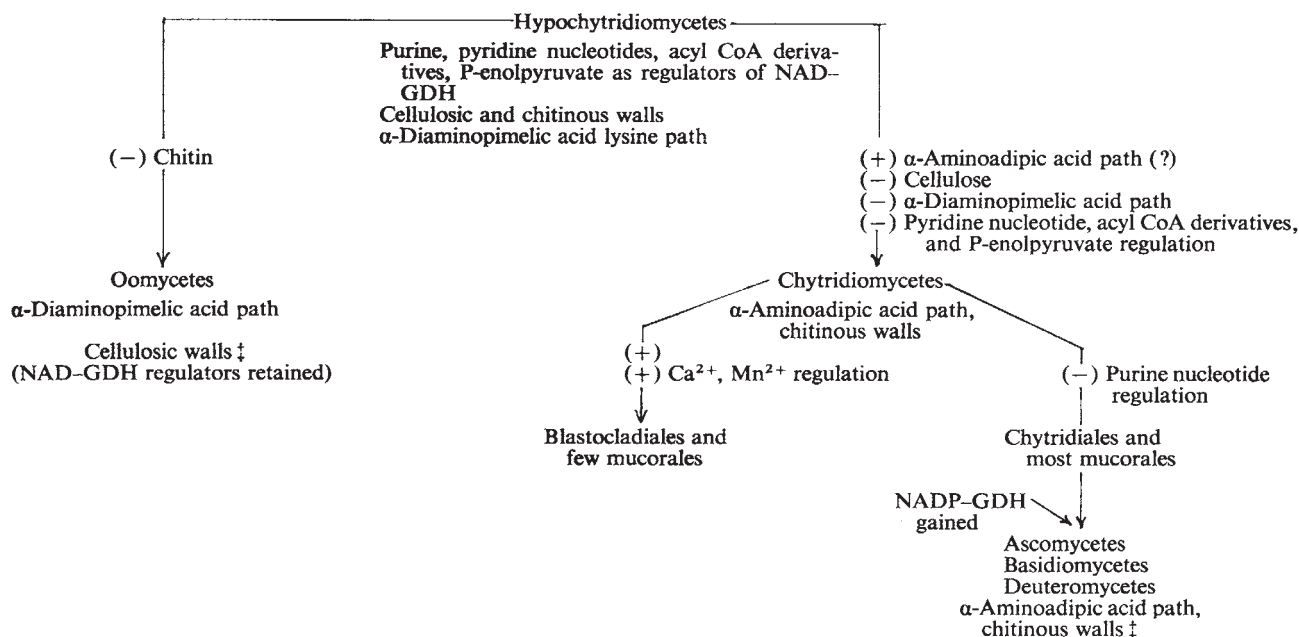


Fig. 1 Suggested path in the evolution of NAD-linked glutamic dehydrogenases in fungi and relationship to cell wall structure, lysine biosynthesis and phylogeny. (+) signifies acquisition of ability, and (-) loss of capacity. The suffix, ‡, means that exceptions are known. Lin and Aronson³² have shown by X-ray studies the coexistence of cellulose and chitin in *Apodachlya*, an Oomycete; chitin and cellulose are also present in *Ceratocystis*, an Ascomycete.

regulatory mechanisms alone would fit the evolutionary pattern presented in Table 2. A relatively unsophisticated enzyme, in terms of control, was gradually modified so that regulatory site(s) for purine nucleotide binding evolved. Further modification led to the evolution of a site that could accommodate pyridine nucleotides (NADP⁺ and NADPH) as allosteric ligands as well. Later, the protein was altered to a form that could use NADP⁺ as a substrate. However, an enzyme of a type that uses both NAD⁺ and NADP⁺ as substrates, although it has been found in higher animals³⁶, has not yet been found in fungi.

A more likely explanation is that the allosteric effectors are indicators of the ancient catalytic history of the enzyme. In ancestral times, the effectors were reactants of primitive "glutamic dehydrogenases". As pathways evolved, some reactants became modifiers on altered forms of duplicated glutamic dehydrogenases. In this way, the enzyme could have evolved from a position of apparent complexity to one of simplicity. Our results, which take into account control mechanisms, cell wall structure and metabolic pathways, support the second concept (Fig. 1). The data on types II and III enzymes and the intermediate form of glutamic dehydrogenases of the Phycomycetes offer a potentially useful system for studies of enzyme evolution.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Circularly Polarized Visible Light from Jupiter

WE have discovered circular polarization of the reflected light from Jupiter, the first detection at optical wavelengths of this type of polarization in light from a planet. The discovery was made at Mauna Kea Observatory, Hawaii, with a photoelastic polarimeter used also in measurements on magnetic white dwarf stars¹. The first results were obtained with the 88 inch telescope on the night of March 31, 1971, and protracted observations were made with a 24 inch telescope on the nights of April 11, 20 and 21.

The appreciable linear polarization of the planets, which varies from planet to planet as well as with the phases and location on the disk, has discouraged a search for any circular polarization, which is expected to be much smaller than the linear component. Every polarimetric system (which includes the telescope optics) has a certain degree of spurious linear-circular conversion. Our apparatus has a linear-circular transformation coefficient of about 10^{-3} at mean visible wavelengths. In the presence of 1% linear polarization, the false circular component is $\sim 10^{-5}$. By averaging against measurements with the polarimeter rotated by 90° , we can measure circular polarization as small as $\sim 3 \times 10^{-4}$ of the linear polarization. On Jupiter the linear polarization is approximately radial on the disk² (at or near opposition phase) with a maximum of about 6% on the limbs at 5000 Å. In this case we can readily detect a circular component as small as 2×10^{-5} and rather smaller values when the system is very carefully aligned. Qualitative agreement (same handedness of the values) between results from the two telescopes served as a check that nothing in the telescope optics was responsible for the signals.

Our measurements were all made in the red at a mean wavelength of 6800 Å with a mean bandwidth of ~ 500 Å. This pass band was defined by using a Corning 2-64 filter (sharp red cut-on) combined with an S-20 photocathode, which has a response falling rapidly in the far red. We selected this spectral region in order to reduce the spurious linear-circular conversion, which is less severe in the red, and because some theoretical mechanisms predict a circular polarization increasing with wavelength. In spite of the filter attenuation, each of the polarization values on Jupiter could be established after 5–10 min of signal averaging on the 24 inch telescope with an aperture diameter 7 arc seconds.

There is a general "polar" effect which is shown in Fig. 1a. The north and south polar regions and, roughly speaking, the northern and southern hemispheres have opposite circular polarizations Q_N and Q_S . The "handedness" in the north is that for an observer facing the planet, and at the orbital phase angles obtaining during the measurements the E vector rotates clockwise; in our convention Q_N is negative. The north and south polarization values are unequal and Q_N was consistently about twice as large as $-Q_S$. The whole planet was also found to have a small net polarization Q_0 , in the "north" sense. Each of the data in Table 1 is an average of several measurements over a period of ~ 4 h on each of the nights. There

seemed to be no strong dependence on the rotational phase of the planet and within our accuracy, Q_N , Q_S , and Q_0 showed no significant changes as the red spot emerged, transited and set behind the west limb.

Table 1 Fractional Circular Polarization Values for the Apertures and Locations of Fig. 1a

Night	Q_N ($\times 10^{-5}$)	Q_S ($\times 10^{-5}$)	Q_0 (whole planet) ($\times 10^{-5}$)
March 31, 1971	~ -10	$\sim +10$	—
April 11, 1971	$-(8.2 \pm 1.5)$	$+(4.0 \pm 1.5)$	—
April 20, 1971	$-(6.1 \pm 1.5)$	$+(2.3 \pm 0.5)$	$-(1.0 \pm 0.3)$
April 21, 1971	—	—	$-(1.5 \pm 0.3)$

One striking local anomaly was seen, but was studied less methodically. On April 21, the region of the red spot was probed with an aperture of 7 arc seconds⁶. The west or leading end of the spot, designated B in Fig. 1b, showed a large Q in the southern sense. The approximate values for two regions near the west and east ends of the red spot respectively were $Q_B = +1.2 \times 10^{-4}$ and $Q_A = +0.3 \times 10^{-4}$. No comprehensive scans have been made but a qualitative probing repeatedly showed a largest positive Q localized perhaps somewhat to the west of the west end of the red spot. No visible features in precisely this position could be found in photographs taken on the same observing night. Observations over a period of more than 1 h showed that the polarization feature seemed to follow the motion of the red spot across the disk.

The circular polarization of the radio emission from Jupiter³, which has been ascribed to a synchrotron mechanism, is not necessarily related to similar effects at optical wave-

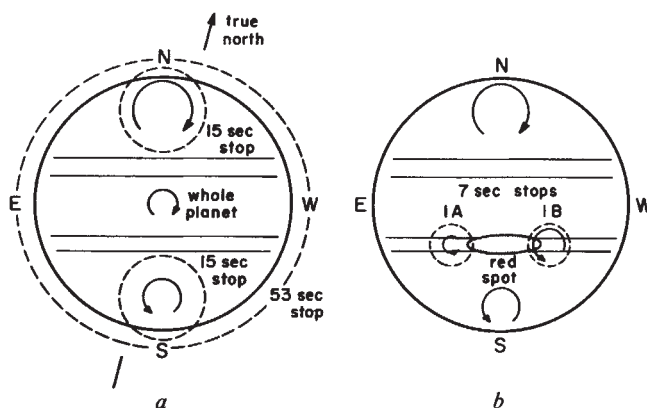


Fig. 1 Circular polarization effects in the reflected light from Jupiter. *a*, The predominant "polar" effect. The dashed circles show the apertures used and the approximate locations; the sizes of the circular arrows show the relative magnitudes of the Q values measured. *b*, The peculiar local polarization feature around the west end of the red spot found in one observation.

lengths. It was tempting, however, to try a magnetic interpretation to the grey body magneto-emission⁴ there is a corresponding magneto-absorption, given (for propagation along the magnetic field direction) by $q \sim \Omega_c/\omega$, where Ω_c and ω are cyclotron and optical frequencies respectively. For reflexion at normal incidence in a simple reflecting layer model there is a magneto-reflectance $Q = 2q(1-R)$, where R is the albedo or reflexion coefficient. For $R=0.7$, the value $Q \sim 10^{-4}$ at visible wavelengths implies a field $B \sim 10^4$ gauss for grey reflectance. But "spectroscopic" magneto-absorption proportional (to first order) to the spectral derivative of the absorption coefficient⁵ might predominate. If the fractional change in absorption (strictly speaking the electronic part of the absorption⁵) were unity over a measuring pass band $\Delta\omega$, we could make the crude estimate $q \sim \Omega_c/\Delta\omega$. In our measurements $\Delta\omega/\omega = \Delta\lambda/\lambda \sim 0.1$, and it is possible that a field as small as 10^3 gauss could produce the necessary magneto-reflectance values. Such a field is still large compared with those often postulated for Jupiter—10 to 100 gauss—in connexion with the radio emission, but no accurate measurements of the field are available⁶. The field would have to have an aspect coinciding at least approximately with the planet's rotation axis to account for the basic polar effect described above.

A more prosaic mechanism⁷ for the polarization is oblique surface scattering and the chief "polar" feature (opposite signs of Q_N and Q_S) is consistent with that model. The differences in magnitudes of Q_N and Q_S could be ascribed to differences in mean absorption, at the measuring wavelengths, between the north and south hemispheres. There is a moderately strong CH_4 absorption band⁸ at 7250 Å but Jupiter hardly exhibits any north-south symmetry in surface gas composition or other properties. But apart from the red spot and smaller features it is a reasonable figure of revolution. The scattering model then predicts that Q_N and Q_S pass through zero and change sign as the planet passes opposition. Our first data (Table 1) already suggest a decrease as the opposition on May 23 is approached.

During May and June we will look for the surmised sign changes in the polar Q values. Though the north-south effect may well be due to the scattering mechanism, it is still possible that the "hot spot" in Q is magnetic in origin. (There should, therefore, be an opposite magnetic pole elsewhere on the planet.) Correlation with the rotation systems I, II and III will be of interest.

The polarization measurements were surprisingly easy for Jupiter and the way is open for a fascinating programme of circular polarization work on other planets. Detection of genuine magneto-reflectivity, for example, would be interesting; and a propitious case for study might be Uranus, whose spin axis lies almost in the plane of the solar system, so that an "end on" magnetic effect could be observed. The non-magnetic polar scattering effect should, however, be very general—it ought to be visible on Venus—and might reveal unexpected facts about planetary atmospheres.

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Elliptical Polarization by Surface-layer Scattering

THERE is a very simple model which exhibits circular, or more accurately elliptical, polarization in light scattered obliquely from a surface and which could be applied to the circularly polarized reflected light from Jupiter¹. The incident light is assumed unpolarized and several features are necessary for such an effect: (a) appreciable absorption, to generate a phase shift (quadrature retardation) between the independent linear components of the scattered light, (b) double or multiple scattering, and (c) oblique geometry, that is, a general and non-coplanar relationship between incident ray, surface normal and scattered ray vectors. Some polarization aspects of multiple light scattering have been discussed in the past^{2,3}, but as far as we know, the type of model described here does not appear in the literature.

The easiest way to see how circular polarization can arise from scattering is to use the notions of momentum conservation. Fig. 1 shows scattering of a stream of photons, unpolarized in the incident state, from the north polar region of a spherical body. The y axis is the surface normal at the scattering point, and xy is the incident plane. If the angle ψ between incident and scattered ray vectors is 90° , the linear photon momentum $p = \hbar k_{\text{inc}}$ along this direction is transferred entirely to the body, which recoils in the plane of k_{inc} and k_{scat} . But this involves also a rotational impulse $L = R \times p$, where R is the radius vector from the body centre to the scattering point and is the lever arm for an impulsive torque. The body is given a twist about an axis through its centre which is in fact parallel to z (that is, to k_{scat}) if the scattering point is at the north pole, but here only the z component of the angular momentum is of interest. Actually the angular momentum L , after scattering, is distributed between the body and the photon (that is, $L \sim L_z = L_z^{\text{phot}} + L_z^{\text{plan}}$ —statistically the photon comes off with a definite angular momentum). The distribution

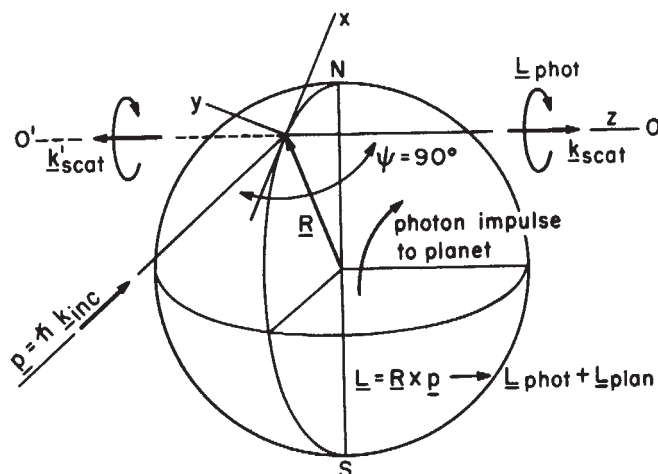


Fig. 1 Geometrical relationships for scattering from a local point on a spherical body. Linear and angular momentum directions for a simple model which predicts circular polarization in the scattering is also shown.

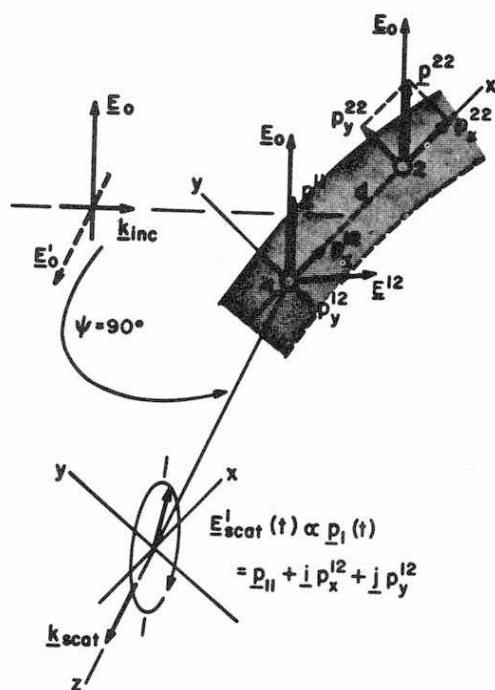


Fig. 2 A two-particle scattering process representing a specific scattering mechanism for Fig. 1. The incident radiation field exerts a torque on the surface layer, which accounts for the angular momentum of the scattered field.

and perhaps even the sign of L_z^{phot} (the "handedness" of the circular polarization) must depend on details of the scattering process, although L_z^{phot} is not generally zero. But the following symmetries are easily deduced: (1) for scattering in the $-z$ direction where $\psi = -90^\circ$, that is, as viewed by observer $0'$ in Fig. 1, the polarized handedness is reversed (supposing, of course, the surface material to be homogeneous and two dimensionally isotropic), and (2) for an equivalent scattering in the southern hemisphere the handedness for scattering along z is again reversed.

In an explicit two particle scattering model (Fig. 2) the xyz axes correspond to those in Fig. 1. A cross section of the scattering surface is shown, which, for illustration, is thought of as an atomic monolayer. It is helpful to suppose that the interior of the body is filled with non-reflecting, totally absorbing material. The atoms or molecules of the scattering layer have, individually, isotropic polarizabilities α , or, if they are non-spherical, the polarization axes are randomly oriented. They are also partially absorbing, so that α is complex. The field E_{scat}^1 radiated along k_{scat} by atom 1 is due to this atom's primary excitation p^{11} (αE_0 where E_0 is the incident light field) plus its secondary excitation p^{12} caused by a nearby excited atom 2, a distance d away. Only the linear component E_0 of E in the incident plane of the incident light need be considered; the other component E'_0 (parallel to z) does not produce primary (single scattering) radiation along z , and also cannot yield circularly polarized light by double scattering along this direction.

Coherence is required, so we consider only interactions for which $d < \lambda$. The primary field E_0 then has approximately the same phase for atoms 1 and 2, and the interaction between these two proceeds by simple electrostatic dipole-dipole coupling. The secondary dipole-field components acting on atom 1 are $E_x^{12} = +2 d^{-3} p_x^{22}$ and $E_y^{12} = -d^{-3} p_y^{22}$, constituting a net field E^{12} ; it is significant that E^{12} is not parallel to E_0 . Because of the absorption, p_x^{22} and p_y^{22} are not in phase with E_0 —they lag behind E_0 by as much as 90° . Similarly the secondary field E^{12} also lags behind E_0 . The total field $E^1(t) = E_0(t) + E^{12}(t)$ acting on atom 1 is thus elliptically polarized as is the excitation $p^1(t) (= \alpha E^1(t))$ and the scattered wave field

E_{scat}^1 . Radiation from atom 2, as scattered first by atom 1, is treated analogously and has the same handedness as that from atom 1.

The pair of atoms 1, 2 was chosen to lie along the x axis, normal to the scattering direction z , but for a pair lying along the z direction, the interaction does not produce ellipticity; such pairs merely reduce the proportion of elliptically polarized light.

A monatomic layer is not essential and represents only an extreme case of surface anisotropy for which all scattering pairs lie in planes tangent to the surface. In a thick layer, for example, a planetary atmosphere, there is evidently still some degree of surface anisotropy in this sense. A photon which has emerged after double scattering is more likely to be scattered by a pair of particles oriented in the surface plane than by a pair normal to it. If after one scattering process a photon travels outwards, it will probably not undergo a second scattering and will leave with zero circular polarization (in the statistical sense), because single scattering cannot cause such polarization; if it travels inward, however, it will probably be completely absorbed.

A specific handedness of the ellipticity follows from this model, and is indicated in Fig. 2. For scattering to the right from the north polar region of the body in Fig. 1, the E_{scat} vector rotates clockwise as viewed by an observer on the $+z$ axis facing the scattering body. This is associated with the phase lag of the dipole moments and with the definite vector directions of the x and y components of the dipole-dipole coupling which are indicated in Fig. 2. This sense is independent of the model parameters d/λ and α , and it may encompass more extended models. The "polar scattering" from Jupiter¹ has indeed the senses predicted here, if account is taken of the orbital phases of the Earth and Jupiter at the time of the reported measurements.

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Interpretation of Extraterrestrial Lyman-alpha Observations

RECENTLY, several new extraterrestrial Lyman- α measurements have been made available from Venera 2, 3 and 4 (refs. 1 and 2), OGO 3 (ref. 3), Vela 7 (ref. 4), Mariner 6 (ref. 5) and OGO 5 (refs. 6 and 7). The two groups operating with the OGO 5 satellite have given so far the most comprehensive analysis of the celestial distribution of extraterrestrial Lyman- α radiation in their sky map of Lyman- α isophotes, which contains an almost complete representation of the directional distribution of Lyman- α background radiation.

All the a.m. observations proved that there exists a significant anisotropy of the Lyman- α background radiation with a pronounced broad spatial intensity maximum and minimum. This feature, and variations in intensity on a time scale of several months, is evidence against the hypothesis⁸ that the source of this radiation is galactic. The very large number of scattering processes which a galactic Lyman- α photon would undergo before reaching the solar system implies that the Lyman- α quanta have lost almost all information concerning their origin and would therefore arrive isotropically at the solar system. Of course, a constant amount of isotropic radiation of galactic origin might very well be contained in the total anisotropic extraterrestrial Lyman- α background.

The source for the principal (anisotropic) part of the observed radiation is, however, interplanetary. The basic idea is that solar Lyman- α radiation is scattered back from neutral hydrogen which has entered the solar system from interstellar space, and the penetration of interstellar hydrogen into the solar system and the resulting Lyman- α scattering has been analysed theoretically⁹⁻¹¹.

The theories of Patterson⁹ and of Hundhausen¹⁰ did not take into account the macroscopic velocity of interstellar hydrogen relative to the solar system. This velocity—which is probably of the order of 20 km s^{-1} —drastically affects the hypothesis, however, as it yields much larger interplanetary hydrogen densities and an anisotropic spatial density distribution. The vector velocity of the interplanetary hydrogen relative to the Sun is its axis of rotational symmetry. Considering these factors, Blum and Fahr^{11,12} have given a quantitative model of the distribution of interplanetary hydrogen and the scattered Lyman- α radiation which would be expected.

The Lyman- α measurements of Chambers *et al.*⁴ were carried out with ultraviolet spectrometers which were always pointed radially outwards from the centre of the Earth. Therefore, only a single intensity trace across the sky could be obtained. The explanation of the intensity distribution resulting from these measurements could very well be scattering of solar Lyman- α by interplanetary neutral hydrogen distributed according to the Blum-Fahr model¹². The maximum of Lyman- α radiation appeared at a right ascension of approximately 270° and a declination of approximately 30° . It was impossible to fix the exact position of the maximum in right ascension and declination simultaneously. The interpretation that the maximum was in the direction of the solar apex motion (r.a. = 270° , dec. = 27°), as expected from the Blum-Fahr model in the simplest case, was not conclusive, because directions with approximately the same right ascension but differing declinations were equally possible.

Barth, who analysed the Mariner 6 measurements, found the maximum Lyman- α intensity in the region of Ophiuchus (r.a. $\approx 255^\circ$, dec. = 5°) which would imply a deviation of about 40° from the direction of the solar apex motion.

The OGO 5 measurements also could not confirm that the maximum intensity was located at the exact position of the solar apex. In fact, temporally varying deviations between 40° and 60° were found. In the first spin-up of OGO 5 on September 9, 1969, the maximum was located near r.a. = 220° and dec. = -15° , or about 50° from the solar apex. The second spin-up on December 15, 1969, permitted only a rough localization of the maximum because of perturbations by the geocorona. It was found approximately at r.a. = 260° , dec. = -10° , or about 40° from the solar apex. On the third spin-up in April 1970, the maximum was obtained at r.a. = 270° , dec. = -20° , or about 50° from the solar apex.

The two research groups^{6,7} that have analysed the OGO 5 measurements have essentially accepted the Blum-Fahr model as the basis for their interpretations of the observed Lyman- α background. In order to explain the deviation of the location of the maximum from the solar apex direction they have followed a suggestion made by us¹¹ that the peculiar velocity of interstellar hydrogen relative to the solar system is not

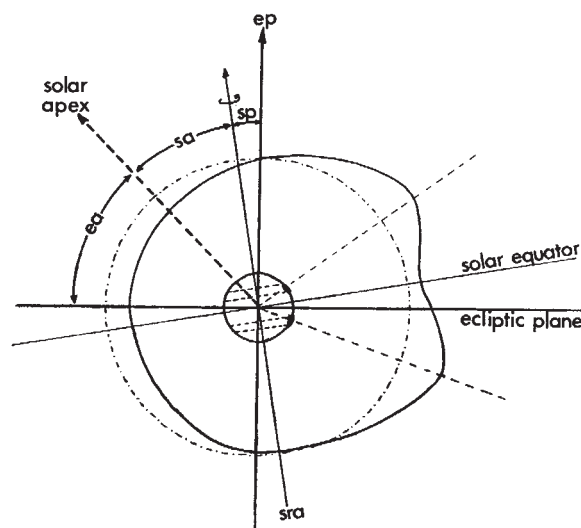


Fig. 1 A schematic representation of the solar Lyman- α intensity pattern in a plane perpendicular to the ecliptic. The angle sp between the direction of the ecliptic pole, ep , and the solar rotation axis (sra) is 7.25° ; the angle sa between (sra) and the solar apex is 37.28° ; and the angle ea (the ecliptic latitude of the solar apex) is 53° . The region bounded by (---) indicates the isotropic undisturbed Lyman- α intensity from the solar disk. Two areas of solar activity are indicated. The dashed line (- - -) shows the direction of maximum radiation from these areas. The full curve represents the total solar Lyman- α radiation with two maxima. This Lyman- α pattern is approximately fixed for several solar rotation periods and co-rotates, therefore, with the Sun in 27 days.

necessarily identical to the negative solar apex motion, but is composed of two parts: the negative solar apex motion, and the peculiar velocity of the interstellar hydrogen medium near the solar system with respect to the local standard of rest. Possible values for the peculiar velocity of the interstellar hydrogen are between 5 and 30 km s^{-1} (refs. 13 and 14) which could, therefore, easily account for a deviation of 45° between the negative apex motion of the Sun (relative to the local standard of rest) and the total motion of the interstellar gas relative to the Sun. This deviation would cause a shift of the Lyman- α maximum from the solar apex by the same angle.

If this were the only reason for the observed deviation of the Lyman- α maximum from the solar apex position, we would not expect any temporal changes in the maximum. The OGO 5 groups^{6,7} have therefore correctly pointed out that a second effect has to be considered. Because of the orbital motion of the Earth, the observations of the Lyman- α backscatter were carried out at different positions within the solar system, although the distribution of atomic hydrogen within the solar system is spatially fixed. This causes a parallax effect when receiving the Lyman- α backscatter from different points of the Earth's orbit.

As well as these two effects, however, another very important effect has not been taken into account, which contributes to a satisfactory explanation of the Lyman- α phenomenon. All interpretations of Lyman- α observations as backscatter of solar Lyman- α radiation have so far assumed that the solar Lyman- α radiation is spherically symmetric and isotropic, which is definitely not the case. Meier¹⁵ monitored the solar Lyman- α radiation and found a 30% intensity variation during the solar rotation period and larger variation was found by Hall and Hinteregger¹⁶. This proves that the source of the solar Lyman- α radiation is to be found both in the undisturbed solar disk and the solar activity centres. The latter are located mostly at moderate solar latitudes and possess a lifetime of several solar rotation periods. They have a fixed position on the Sun and therefore co-rotate with the solar surface. The radiation from a solar activity centre for the 10.7 cm radio region, the Lyman- α line and the EUV region must be assumed to be emitted chiefly

in the direction normal to the solar surface at the position of the centre. We might expect from this reasoning that the solar Lyman- α radiation will show strong asymmetries related to heliographic longitude and latitude. A theoretical estimate of the Lyman- α radiation background has therefore to take both the anisotropy of the interplanetary hydrogen distribution and the anisotropy of the exciting solar Lyman- α radiation into account. An example of this effect is given in Fig. 1. The periodically changing intensity of the solar Lyman- α -radiation must also contribute to the temporal intensity variations of the background radiation. As well as the experimental difficulties resulting from calibration errors and instrumental deterioration, this effect might also contribute to the diverging results of the absolute maximum intensity of the Lyman- α background found.

A further physical complication results from the varying Lyman- α radiation pressure. As this counteracts the solar gravitation in determining the orbits of interplanetary hydrogen atoms, the overall effect would be an attraction towards the Sun which depends both on distance from the Sun and on time. Therefore, the resulting orbits will not be purely Keplerian, as assumed in the original model of Blum and Fahr, but will deviate, which, in turn, will cause further anisotropies in the interplanetary hydrogen distribution.

Furthermore, the changing EUV radiation within the solar rotation period also has some effect on the density pattern of the interplanetary hydrogen. This influence has already been studied quantitatively¹⁷. Its effect on the Lyman- α scattering is, however, only a few per cent.

We are investigating all the various effects mentioned here. Preliminary computational results have revealed that the shift in the direction of the radiation maximum because of the asymmetry of the solar Lyman- α radiation becomes of the same order of magnitude as the shift caused by the parallax effect, if 30% deviations in the solar Lyman- α radiation in specific directions (as explained here) are assumed.

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Galactic Spurs as Possible Sources of Soft X-radiation

SOFT thermal X-radiation from the Cygnus Loop has been discovered recently¹. One of us² has predicted the existence of such radiation from supernova remnants on theoretical grounds. It is possible that the source Vela X-1 has a similar origin³.

At radio frequencies, features in our galaxy such as the North Polar Spur have been known for more than 20 yr, and include in the southern hemisphere the Cetus Arc⁴ and in the northern hemisphere the object known as Loop III⁵. In 1960 the hypothesis was advanced that the North Polar Spur is the remnant of a supernova outburst that occurred in the close vicinity of the Sun, at a distance of 20–30 pc (ref. 6).

If this hypothesis is true we can expect the presence of soft X-radiation at a significant level of intensity in the spur region, because the surface brightness of spurs in the radio band is comparable with the surface brightness of the Cygnus Loop. Note that one should not expect detailed correlation between the radio and X-ray brightnesses. In the case of the Cygnus Loop there is an indication of a shell structure to the X-ray source and a general correlation with the system of observed filaments, but the correlation with the radio image is less good.

We wish to compare the results of some observations of the soft X-radiation background in the range of 44–60 Å with the distribution of spurs over the celestial sphere. We took a figure that shows the results of scanning the celestial sphere in the soft X-ray region of the spectrum⁷, and plotted the main ridges in the distribution of brightness temperature of the galaxy's radio background (at a frequency of 240 MHz) that conform with the North Polar Spur⁸ and Loop III⁵ in the northern hemisphere, and with the Cetus Arc⁴ in the southern galactic hemisphere. The broken line on Fig. 1 represents scans over the celestial sphere from which the distribution of X-ray background in the 44–60 Å band has been investigated.

There is a definite dependence of the soft X-radiation background on galactic latitude^{7,9}. This anisotropy of background is explained by absorption of X-radiation of extragalactic origin by galactic hydrogen and helium. Some authors, however, have failed to find systematic variations of this kind in the X-ray background^{10,11}.

Measurements of the soft X-radiation background showed that the intensity of radiation at 0.27 keV is considerably higher than the background value obtained as a result of extrapolation of a power spectrum with index—1.5 from higher energies. This surplus radiation in the range 44–60 Å is explained by thermal bremsstrahlung from intergalactic gas with a temperature of the order of 10⁶ K. The optical thickness of galaxy for radiation of 0.27 keV is about 1. But according to refs. 7 and 9 measurements of the soft X-ray background variation with galactic latitude imply that the number of hydrogen atoms on line of sight must be less than expected from the measurements at 21 cm. An attempt to explain the absorption decrease by assuming that hydrogen is collected into clouds with density of 10²¹ atoms cm⁻² does not seem to be valid⁹.

Our conjecture is that the increase of radiation intensity in the range of 44–60 Å coincides with the spur regions. The variation of background intensity near the Cetus Arc correlates better with the position of the optical filaments¹² that cover the radio structure of the Cetus Arc.

Although the detailed traces have not been presented in refs. 10 and 11, it follows from these letters that the scanning was performed over the regions where the peculiarities in radio frequency radiation related with galactic spurs are absent. This fact might explain misunderstandings which have arisen in the interpretation of observational data by different groups^{13,14}.

New observations are required for solution to the question of the connexion of the X-ray background in the soft region of the spectrum with radio objects like galactic spurs. In the region of even more soft X-radiation, 80–100 Å, one should also expect the similar variation of background intensity, although somewhat weaker. Our galaxy is completely non-transparent to extragalactic radiation in this region of the spectrum¹⁵. Such measurements would be the deciding factor in the verification of the validity of this hypothesis.

It is probable that soft X-ray emission observed at low galactic latitudes can be explained as an integral effect from

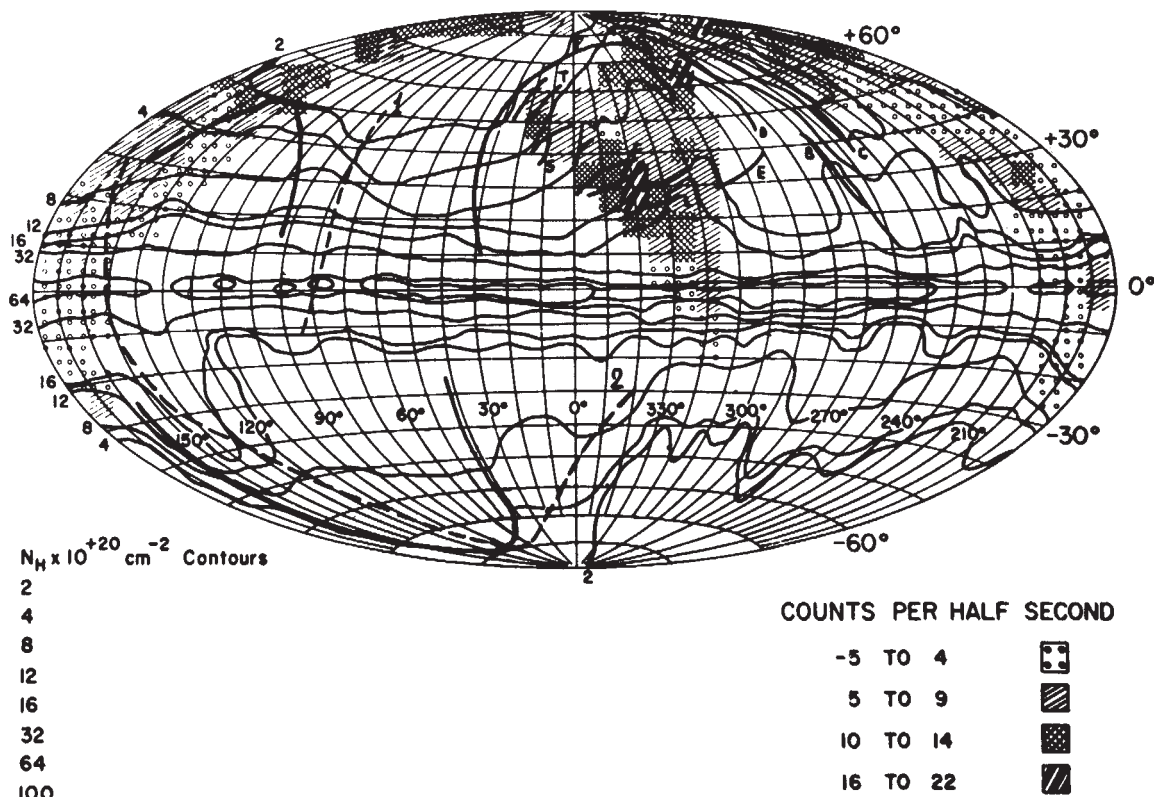


Fig. 1 Distribution of the X-ray background intensity in the range of 44–60 Å and distribution of neutral hydrogen in galactic coordinates⁷. The main ridges of radio spurs are shown by solid curves. Letters denote the fine details of North Polar Spur radio structure⁸. Dashed lines are the traces of X-ray detectors over the celestial sphere: curve 1 according to ref. 10 and curve 2 according to ref. 9.

old remnants of Type II supernovae, while the similar emission from high galactic latitudes is caused by such objects in the immediate neighbourhood of the Sun (spurs). The recent discovery of an emission line in region 0.27 keV which was observed from low and high galactic latitudes (unpublished results of M. Lempton and others) evidently supports our proposal.

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Radon-222 Emanation and the High Apparent Lead Isotope Ages in Lunar Dust

URANIUM-LEAD and thorium-lead ages for Apollo 11 and Apollo 12 lunar samples do not match and, furthermore, the dust samples seem to be older than the rocks. These experimental observations are generally thought to require some source of parentless lead¹.

We propose that the escape of ²²²Rn, ²¹⁹Rn and ²²⁰Rn produced in the ²³⁸U, ²³⁵U and ²³²Th decay chains may be a significant mechanism for a partial redistribution of the post-radon lead daughters. Kraner² suggested that there may be an alpha-emitting radioactive deposit on the lunar surface as a result of the decay in space of radon isotopes which might diffuse out of lunar surface material. Turkevich³ reported evidence for such an alpha-emitting deposit in Mare Tranquillitatis, although no such evidence was found at Sinus Medii or outside the crater Tycho.

Preliminary experiments have demonstrated that ²²²Rn readily escapes from both lunar and terrestrial materials. Using a non-destructive technique and radon detectors developed at Argonne National Laboratory⁴, the ²²²Rn emanation from a portion of Apollo 12 sample 12070 was found to be 7.4×10^{-18} Ci s⁻¹ g⁻¹ at room temperature and a pressure of 1 atmosphere. The sample used consisted of 473 mg of 12070 dust from which the fraction larger than 60 μm (40% by weight) had been removed for other inert gas experiments. The uranium concentration in 354 g of sample 12070 was determined to be 1.5 ± 0.2 p.p.m. using gamma-ray spectrometry⁵. Assuming this concentration for the sample used in our experiment, the ratio of the emanation rate of ²²²Rn to the production rate is 0.48 (that is, 48% of the total amount of radon produced escapes from the material). Because of the small amount of sample used, the statistical accuracy of this measurement was poor; however, the accuracy is estimated to be better than 50%.

This result indicates that there is a substantial migration of ^{222}Rn (half-life=3.825 days) above and below the lunar surface, and there may also be significant migration of ^{220}Rn (half-life=54.5 s) and ^{219}Rn (half-life=4.0 s). Assuming that the radon gas is thermalized by the time it escapes from the lunar material, we calculate a velocity of hundreds of metres per second, so that even the short-lived ^{219}Rn could get very far from its point of origin. It is clear that the different half-lives will result in isotopic fractionation which may have an important influence on the apparent uranium–thorium–lead ages.

There are a number of factors which these experiments did not include. Among these are the dependence of the emanation rate on pressure and temperature and the effects of the solar wind, magnetosphere, and light pressure. It should be noted that half of the daughters of the radon atoms which decay in space above the lunar surface will escape from the Moon because of the high recoil velocity of the daughters. The remaining half will be implanted on the lunar surface and subsequently decay to the stable lead isotopes ^{206}Pb , ^{207}Pb and ^{208}Pb . We think that the emanation rates of the radon isotopes should be measured for all types of lunar samples in order to determine the importance of this phenomenon.

The lunar sample analysed non-destructively was obtained from NASA. We thank Professor D. Heymann and Dr A. Yaniv for discussions. This research was supported by the International Atomic Energy Agency (fellowship to P. M. B.), the Department of Defense (fellowship support to R. B. C. and J. S. D. and experimental apparatus), NASA (apparatus) and the Robert A. Welch Foundation.

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Structure of Glassy Carbon

WHEN many polymers are pyrolysed, they change directly into a form of carbon which retains the original morphology without passing through a plastic phase. This type of carbon has a glass-like appearance and is referred to as a glassy carbon. It is hard and brittle, unlike the soft graphitic forms of carbon, and does not revert to these forms at high temperatures.

The lack of information about the starting materials and the carbonization process given by manufacturers precludes the elucidation of the structure of the material; it is necessary to study carbonization mechanisms in well-defined starting materials.

We have previously shown that glassy carbon can be prepared from a phenolic resin in the shape of disk and fibre by a simple process^{1,2}, and we have used this material to establish the microstructure. The carbonization mechanisms have been analysed by X-ray diffraction, infrared spectroscopy, hardness, Young's modulus and tensile strength.

The results indicate that the principal carbonization mechanism is the formation of intermolecular cross-links between hydroxyl groups in phenolic nuclei and methylene bridges

connecting phenolic nuclei with elimination of water between 350 and 500° C. This produces local coalescence of the chain molecules and the formation of aromatic ribbon molecules. The overlap of the aromatic ribbon molecules gives an X-ray diffraction band which is similar to that observed in carbonized coals³. At this stage, the structure consists of long, narrow and imperfect aromatic ribbon molecules which are randomly oriented and tangled in a complicated manner.

X-ray diffraction studies confirm that the ribbon network structure of the material heat-treated at 500° C is essentially preserved throughout subsequent high temperature treatment. At more than 500° C, the ribbon molecules approach each other with the elimination of hydrogen, accompanied by large dimensional shrinkage as intermolecular cross-links are formed between ribbon molecules: there is a rapid increase in hardness and Young's modulus with heat treatment, and this process is complete at 1,500° C.

We consider that the final glassy carbon has a network structure consisting of tangled aromatic ribbon molecules which are cross-linked by highly strained carbon–carbon covalent bonds with a wide spectrum of bond energies.

At more than 1,500° C, the modulus and hardness decrease as L_a increases. We attribute this to the flattening and straightening of ribbons and ribbon stacks between boundaries which become more localized and definite as the "graphitization" proceeds; this process is like polygonization in metals. This would involve the progressive elimination of highly strained ribbon–ribbon bonds, leaving only those which are thermally stable at progressively higher temperatures.

The development of parallel stacked extensive sheets of graphite (which is a characteristic of a truly graphitizable material) is prevented because continuity is preserved along the length of each ribbon by strong C–C bonds, which would have to be ruptured to produce extensive areas of graphite sheet. The inter-ribbon C–C bonds are much weaker, presumably because adjacent *p*-orbitals do not overlap to the maximum extent, but are highly strained and have a range of bond strengths. A large proportion can be broken at more than 2,500° C; the visco-elastic behaviour at these high temperatures enables lengths of ribbon to move past each other with relative ease (D. B. Fishbach, private communication). Permanent alignment of these stacks of ribbons is possible by pulling isotropic glassy carbon fibres at these high temperatures⁴: Ruland⁵ considers that high modulus carbon fibres consist of aligned microfibrils comprising stacks of ribbons.

This theory for the development of a glassy carbon structure has been deduced from the results of carbonization studies; direct evidence of the structural features thus predicted has not so far been available. Recently, high resolution electron microscopy studies have been applied to various carbonaceous materials⁶, and the results on our materials support the structural features deduced here.

Fig. 1 shows a high resolution electron micrograph of the material heat-treated at 900° C, revealing short-range order with groups consisting of two or three imperfect parallel layers which are randomly orientated. The wrinkled layers indicate a highly strained structure, the internal strain energy of which cannot be released easily by simple heat treatment because the carbon ribbons are cross-linked by strong covalent bonds.

Fig. 2 depicts the structure of the system heat-treated at 2,700° C. A network of stacked graphite-like ribbon molecules with regions of perfect linearity is clearly visible. The height of the stacks is estimated to be ≈ 40 Å, which agrees with the L_c value calculated from X-ray line broadening.

The graphite-like stacks do not have the discontinuities at "crystallite" boundaries which were once considered to be present in all carbons. The physical significance of the crystallite diameter L_a , calculated from X-ray diffraction line broadening, is thus different from that originally suspected⁷. It is possible, however, to estimate the length of straight or relatively strain-free parts of the graphite-like molecules to be about 100 Å, which is comparable with L_a values calculated

from X-ray line broadening. Therefore, the calculated apparent crystallite diameter has the same physical significance as that of "defect distance" proposed by Ergun⁸ in characterizing the structure of well oriented carbon fibres.

Fig. 3 illustrates a schematic structural model for the network of ribbon stacks in glassy carbon. It is clearly very different from that proposed by Franklin⁹ for non-graphitizing carbons.

The formation of the network structure necessarily entails the formation of micropores because of inefficient packing in an isotropic assembly of ribbons, resulting in a low density of only 1.45, compared with 2.2 in single crystals of graphite.

The nature of the intermolecular cross-links is still unknown. X-ray radial distribution studies¹⁰ suggest that glassy carbon has non-trigonal carbon atoms which could suggest non-

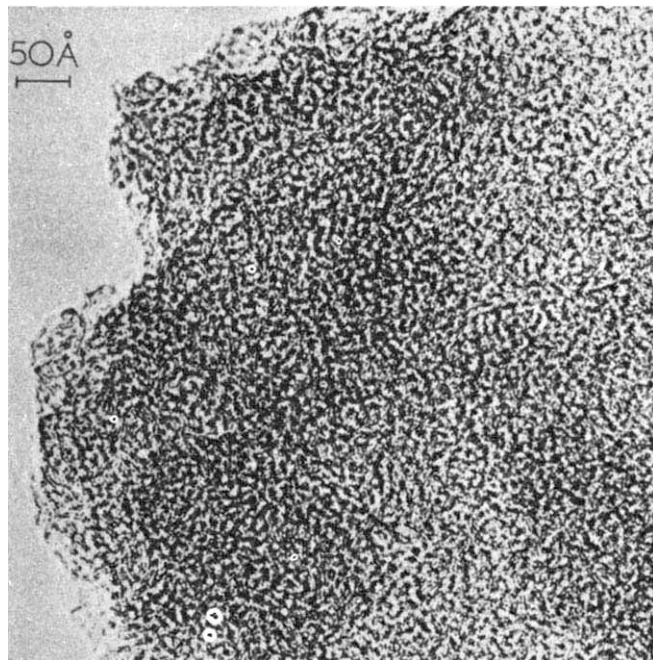


Fig. 1 High resolution electron micrograph of material subjected to 900° C.

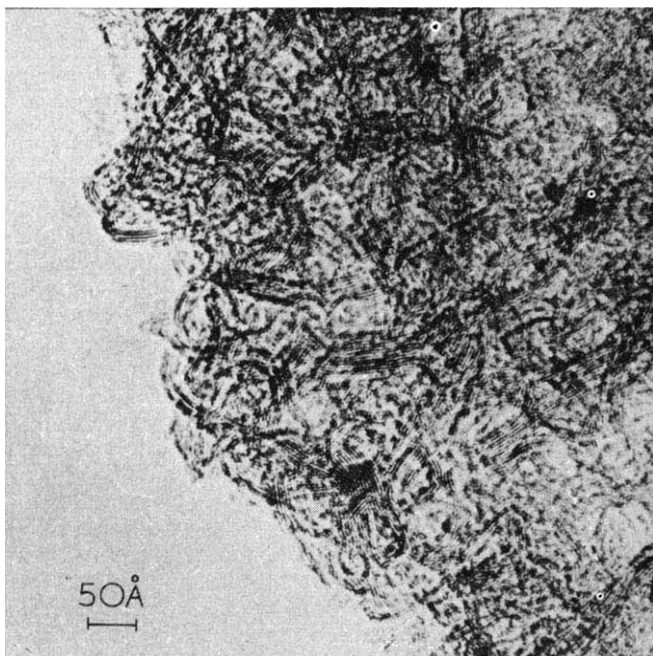


Fig. 2 The structure of the system heated to 2,700° C.

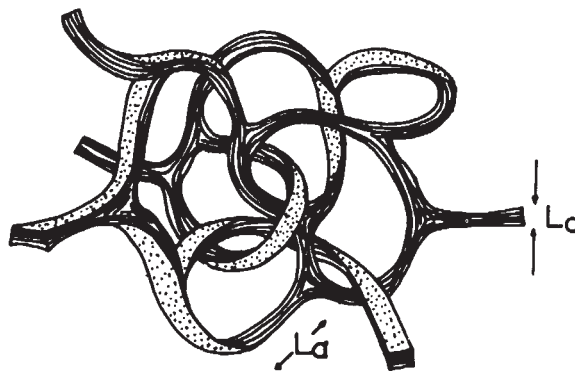


Fig. 3 Structural model for the network of ribbon stacks in glassy carbon.

planar bonding, but this interpretation has been effectively criticized by Ruland¹¹. It is difficult to determine the nature of the cross-links from the results of bond length measurements because the length of a trigonal carbon-carbon bond varies in a condensed aromatic molecule, depending on its size and shape¹² (especially in a highly strained structure). We infer from our results that all the carbon atoms are in the trigonal state after treatment at temperatures greater than 1,500° C and that the bonds between adjacent, rather than parallel, ribbons are rotated and much weakened σ bonds.

A more detailed discussion on these problems will be published later, in connexion with the formation and structure of carbon fibres.

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Trace Element Geochemistry of North Atlantic Aeolian Dusts

WIND-TRANSPORTED (aeolian) dusts make a significant contribution to the land-derived material of some deep-sea sediments¹⁻⁴ and this is particularly significant in the region of the North Atlantic between about 10° N and about 35° N. This region underlies the path of the north-east trade winds which have crossed the Sahara Desert area, and which contain one of the highest reported dust-loadings found in the marine atmosphere⁵. These dusts are important in introducing trace elements into the oceans in association with solid material.

Table 1 Trace Element Content of the North Atlantic Aeolian Dusts

Collection area	Dust sample *	Potential source area	Trace element contents (p.p.m., except Fe ₂ O ₃ which is given in wt. %)									Total carbonate (wt. %)
			Mn	Fe ₂ O ₃	Cu	Ni	Co	Cr	V	Ba	Sr	
51° N–26° N	M14	UK mainland	4,700	17	150	29	12	330	500	560	440	13
	M19	European and North African mainland	1,600	13	340	66	6	180	210	1,400	150	<10
Average, 51° N–26° N	M11	US mainland(?)	365	9	36	50	<5	150	210	1,150	155	N.D.
			2,222	13	175	48	8	220	307	1,037	248	—
31° N–33° N	M20	Deserts,	1,500	12	160	23	7	160	110	850	220	13
	M21	mountains	2,200	13	170	30	<5	140	190	1,100	170	15
	M23	and coastal	2,500	14	250	25	<5	150	100	960	150	<10
	M24	strip of	1,400	23	410	24	17	200	160	2,100	270	14
	M25	Morocco	1,800	18	470	20	12	260	220	1,200	180	<10
Average, 31° N–33° N			1,880	16	292	24	9	182	156	1,242	198	—
28° N–30° N	M27	Spanish	970	16	300	17	7	110	70	890	170	10
Average, 28° N–30° N	M28	Sahara	1,000	13	360	21	12	140	60	900	110	12.5
			985	14.5	330	19	9	125	65	898	140	—
18° N–26° N	M3	Sahara	620	9	53	16	6	71	46	420	335	31
	M4	Desert	365	7	74	108	8	107	540	543	105	<10
	M6	coast of	3,750	7.5	105	50	18	130	240	710	170	<10
	M7	Mauritania,	410	9	118	62	56	83	236	680	150	<10
	M8	long-shore	1,700	7	157	67	10	120	200	760	180	<10
Average, 18° N–26° N	M10	winds	720	8	130	38	23	300	200	660	380	<10
			1,261	8	106	57	20	135	244	629	220	—
17° N–18° N	M1	Sahara	1,200	7	88	50	11	115	153	540	235	20
Average, 17° N–18° N	M2	Desert	1,250	6	91	38	26	115	110	880	160	39
			1,225	6.5	89	44	18	115	131	710	197	—

* For precise locations, see ref. 4.

N.D., Not determined.

Few analyses^{3,6} are available of the trace element content of aeolian dusts, however, none of which give an overall estimate of the trace element content of dusts in the north-east trade winds of the North Atlantic. This report presents the preliminary results of a study of the trace element composition of dusts collected from the north-east trades, the north-east trades-westerlies boundary region and the westerlies in the western North Atlantic in the area between about 17° N and about 55° N. As well as a series of trace elements, analyses are also given for the geochemically important elements iron and manganese.

The collection details, dust loadings, particle size and mineralogy of the dusts have been given elsewhere⁴. One of these publications⁴ described the collection of dust from the marine atmosphere in the north-east trade winds adjacent to the Sahara Desert coast of West Africa. Another (R. C. and L. R. J., in preparation) describes dust collections made off the coast of Morocco, in the north-east trade winds, and off the Iberian peninsula in variable westerlies. An important conclusion was that in offshore winds the dust loadings adjacent to Morocco averaged 3.7 µg m⁻³ of air compared with 10.5 µg m⁻³ of air in similar winds off the West African Sahara coast. Trace element analyses of the dust samples were carried out by a standard spectrographic technique, determining Mn, Fe, Cu, Ni, Co, Cr, V, Ba and Sr. The total carbonate content of the dusts was determined by dissolution in 25% acetic acid, and by an infrared technique. The trace element content of the dusts is given in Table 1, together with the potential dust source areas. On the basis of these source areas, the dusts are divided into groups and the average trace element content of each group is also given. The average trace element composition of all the dusts is compared with those of near-shore sediments and Atlantic deep-sea clays in Table 2.

The principal trends in the trace element geochemistry of the North Atlantic aeolian dusts are summarized as follows. (i) Dusts with a source area in the Moroccan interior contain more Fe₂O₃, Cu and Ba and less Ni and Co than those from the southern Sahara Desert. (ii) The average Ni and Co contents of the dusts are similar to those of near-shore sediments, but are considerably lower than those of Atlantic deep-sea clays. (iii) V and Sr are present in approximately equal amounts in the dusts, near-shore sediments and Atlantic

deep-sea clays. (iv) The dusts contain more Fe₂O₃, Ba and Cu and slightly more Cr than both near-shore sediments and Atlantic deep-sea clays. (v) The Mn content of the dusts lies between those of near-shore sediments and those of Atlantic deep-sea clays. (vi) The high concentrations of Cu, and in certain samples Cr and V, suggest that some contamination of these elements has occurred (see ref. 3). (vii) The aeolian introduction of land-derived material to the oceans may be significant in an understanding of the trace element geochemistry of deep-sea sediments from some areas. A characteristic feature of deep-sea sediments is that they have a higher concentration of certain trace elements, such as Mn, Ni and Co, than near-shore and continental sediments: also in the Atlantic Ocean, the highest concentrations of Mn, Ni and Co are found in mid-ocean areas⁸. The origin of these "excess" trace elements is still unclear; in particular, it is not known whether their direct removal from seawater is more important than their removal in the river environment by particles which are subsequently transported to deep sea areas by oceanic circulation patterns.

In some oceanic areas, for example, that portion of the Equatorial Atlantic which underlies the north-east trade winds

Table 2 Average Trace Element Content of North Atlantic Aeolian Dusts, Near-shore Sediments and Atlantic Deep Sea Clays

Trace element	Average, North Atlantic dusts *	Average, near-shore sediments †	Average, Atlantic deep sea clays ‡
Mn	1,558	850	3,982
Fe ₂ O ₃	12	—	8
Cu	192	48	115
Ni	41	55	79
Co	14	13	39
Cr	160	100	80
V	197	130	173
Ba	905	750	296
Sr	207	250	228

Figures are given in p.p.m., except for Fe₂O₃, which is given in wt. %.

* This report.

† Data from Wedepohl⁷.

‡ Data for Mn, Cu, Ni, Co and Cr from Turekian and Imbrie⁸; data for Fe₂O₃, V, Ba and Sr from Chester and Messiha-Hanna⁹.

Table 3 Average Manganese, Nickel and Cobalt Contents of Sahara Desert Dusts and Mid-ocean Atlantic Sediments

Trace element	Average of dusts originating in Sahara Desert *	Average of <2 μ m fractions of Sahara Desert dusts †	Average of mid-ocean sediments ‡
Mn	1,647	1,300	5,912
Ni	37	30	418
Co	16	15	138

Contents are given in p.p.m.

* Data from Table 1, expressed on a carbonate-free basis for those dusts containing >10% total carbonate.

† The average for the <2 μ m fractions of dusts M1, M2 and M24; the data include any carbonates present in the <2 μ m fractions.

‡ Data from Chester and Messiha-Hanna⁹. The analyses, which are expressed on a carbonate-free basis, are for the following sediment cores: J4 (37° 22' N, 38° 47' W); I4 (34° 06' N, 38° 55' W); H4a (31° 02' N, 45° 13' W); F5 (24° 56' N, 39° 30' W).

between about 10° N and about 35° N, aeolian dusts make up a large proportion of the land-derived material in the deep-sea sediments^{1,4}. The principal source area of these dusts is the Sahara Desert of West Africa. Table 3 lists the average Mn, Ni and Co contents of aeolian dusts from this source, and compares them with mid-ocean sediments in the Sahara Desert latitudes. The average Mn, Ni and Co content of the <2 μ m fractions of some of the dusts is also included as it is these small particles which will be transported farthest from the land masses. Table 3 shows that when bulk compositions are considered, the aeolian dusts cannot supply sufficient Mn, Co and Ni to account for the enrichment of these metals in mid-ocean sediments. If these dusts constitute a large proportion of the land-derived material in the deep sea sediments of this area¹, then at least some of the "excess" Mn, Ni and Co in the sediments must originate from seawater. One possibility is that the dusts are capable of adsorbing trace elements from seawater. This problem is being investigated in our laboratories.

The data now available do not, however, permit the overall importance of aeolian dusts on oceanic trace element economy to be determined. Before this can be evaluated, it is necessary to estimate the efficiency of the dusts as trace element adsorbents and, equally important, to compare the rates of sedimentation of the dusts (and their associated trace elements) with those of the land-derived fractions of deep sea sediments. These factors must be evaluated in terms of the supply of dissolved trace elements to the Atlantic Ocean.

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Thermohaline Staircase

THE use of salinity-temperature depth (STD) systems to study the Mediterranean outflow in the North-East Atlantic^{1,2} has recently drawn attention to the complexity of the thermohaline properties of this mass of water. Our contributions^{3,4} have been concerned with a "step-like" structure in temperature and salinity found within the lower layers of the Mediterranean water from 1,200 to 1,800 m. From August to September 1970, the Liverpool University Oceanography Department conducted an extensive investigation of this phenomenon and some preliminary results are reported here.

One aim of the work was to delineate the horizontal extent of the step-layer zone. Fig. 1 shows the area of operation and the station positions where this type of stratification had previously been observed. Our new measurements revealed an extensive zone (shaded in Fig. 1), which was somewhat further south than had been expected. The western, southern and eastern limits were established by a network of STD stations based on a 10–15 mile grid, but there was insufficient time for a full investigation of the northern limit, which is therefore represented by a broken line on the chart. The results showed that the step layers were distributed over an area of at

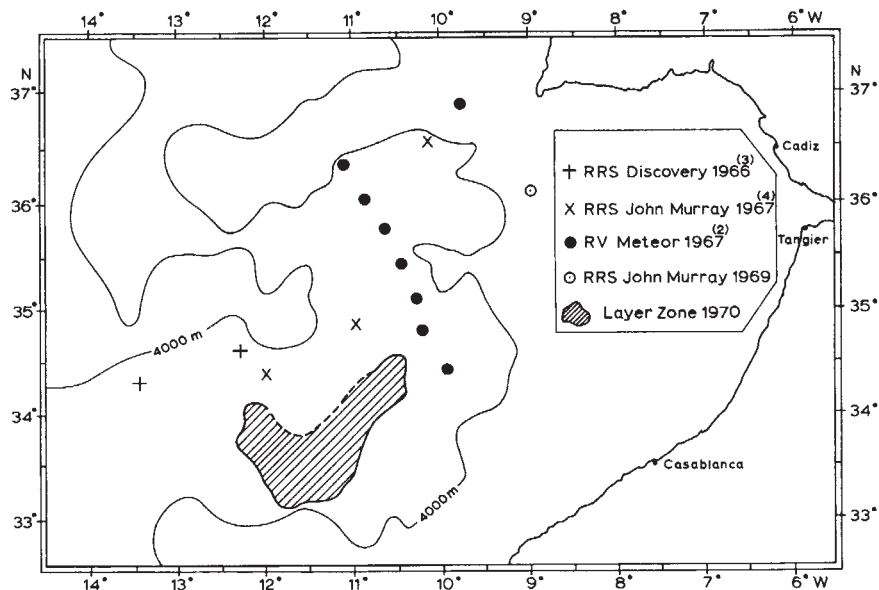
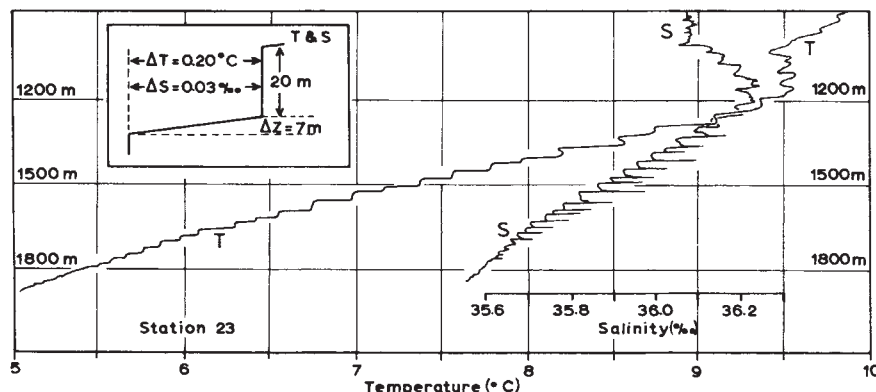


Fig. 1 The location of the layer zone.

Fig. 2 Part of the STD record for station 23: the sharp spikes to the right of the salinity trace are instrumental and can be ignored.



least 4,000 square miles, the boundary of which could be located to within a few miles. More than 150 STD records, usually to depths of 2,000 m, were obtained and all those from stations within the zone showed ten or more well defined step layers. Previously, a maximum of eleven consecutive steps had been reported², but many of the new records showed more than twenty steps forming a thermohaline "staircase" through the lower layers of the Mediterranean water. As well as the standard STD records, expanded scale traces, with a ten-fold increase in resolution, were obtained for all stations.

A typical standard record is given in Fig. 2 which depicts the overall T and S stratification at station 23 (33° 10' N, 11° 46' W) from 900 to 1,900 m. Analysis of the basic parameters from the high resolution traces for this and similar stations produced mean values which can be represented (insert to Fig. 2) as a single step with a layer thickness of 20 m, an interface thickness of 7 m and a decrease in T and S across the interface of 0.20°C and 0.03‰, respectively. Stability is maintained with an increase of 0.005 in σ_t . These values are generally consistent with our previous results. The step and interface thicknesses and the magnitudes of ΔT , ΔS and $\Delta \sigma_t$ all decreased significantly with depth. The interface gradients of T, S and σ_t were more uniform but there was some indication that maximum values occurred through the middle section rather than at the upper or lower extremes.

A primary objective of the cruise was to investigate the horizontal coherence of the stratification, that is, to determine how far a particular layer could be traced by its T/S characteristics before losing its identity. The analysis so far has shown that individual horizontal layers may extend for at least 30 miles. This result is based on data from closely spaced stations at 1 mile intervals as well as more widely spaced stations. For example, the correlation of station 23 with adjacent stations 15 miles away gave T and S values for individual layers which agreed, on average, to within 0.02°C and 0.001‰, an order of magnitude less than the interface values, which leaves little doubt as to the continuity of the layer system.

Over the entire lower boundary of the Mediterranean water outflow, the physical conditions are favourable to the differential diffusion of heat and salt, which has been cited⁵ as the basic mechanism governing the formation of the step layers. The results described here should provide a critical test of any theories concerned with this phenomenon and may stimulate further work. The reason for the development of the layers within a specific area is still unknown, but we hope that the answer will be provided by a complete analysis of the new data. Further results will be reported in due course.

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Depth Distribution in Ocean Basins and Plate Tectonics

IN this article I shall show that relationships between the movement of lithospheric plates¹⁻³ and the depths of the sea floor are leading towards a quantitative theory of the distribution of oceanic depths, and that some predictions can be made. Several principal lithospheric plates have now been recognized; their relative motion over the mantle is described by a rotation of one plate relative to an adjacent plate⁴⁻⁶. The rotation requires two parameters to locate the pole of relative rotation, and one to specify the magnitude of the angular velocity. The direction of spreading is along small circles concentric about the pole of rotation and the velocity of spreading varies as the sine of the distance (measured in degrees of arc) from that pole, to a maximum at a distance of 90° along the equator of rotation. The angular velocity of rotation is the same everywhere. In the Atlantic Ocean the fracture zones between about 60° N and 10° S are very nearly small circles centred about a pole near the southern tip of Greenland (62±5° N, 36±2° W), and the spreading rates approximately agree with the velocities required for the opening of the North Atlantic about this pole^{1,3}.

Examination of the topography of the sea floor and spreading rates in the central region of the world system of mid-ocean ridges shows that the width of the ridge⁷, the local topography⁸, and the thickness of layer 2 of the oceanic crust⁸ seem to be related to the spreading rate in the following way. (1) Slow spreading (1-2 cm yr⁻¹) away from the ridge centre is associated with a narrow ridge, a central rift, adjacent rift mountains and a thick layer 2. (2) Fast spreading (3-4.5 cm yr⁻¹) is associated with a wide ridge, subdued topography (no central rift) and a thin layer 2. (3) The volume of lava discharged in layer 2 per unit time and unit length along the crest of the whole active system is relatively constant regardless of the spreading rate. Thus, topography and the thickness of layer 2 can be predicted if the rate of spreading is known.

An examination of topographic profiles perpendicular to various sections of the world mid-ocean ridge system supports the inference that topography is a function of spreading rate⁹. The relationship between the slope of ridge flanks and the spreading rate from the ridge crest to magnetic anomaly No. 5 at a distance corresponding to 10⁷ yr was formulated from this series of profiles so that, by knowing the spreading rate, the slope can be calculated⁹. The faster the spreading rate within an episode of spreading, the lower the topographic slope and roughness, measured over a distance corresponding to the crust generated during that episode. The decrease in

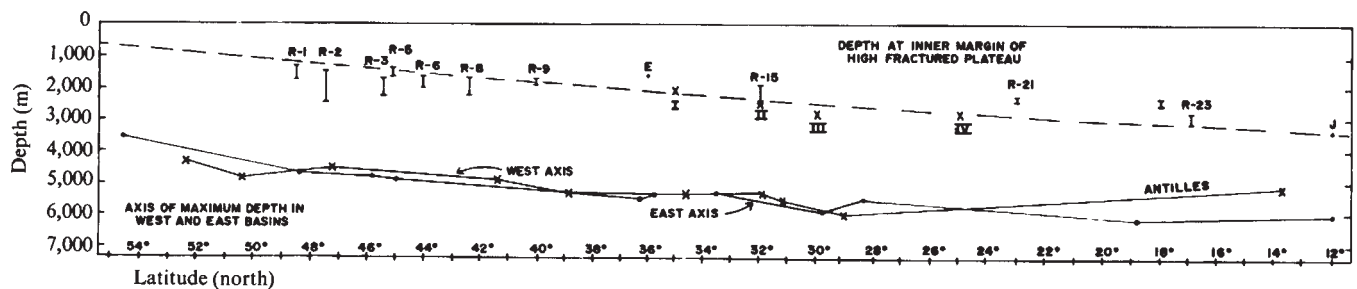


Fig. 1 Depth distribution at the inner margin of the High Fractured Plateau province and marginal basins¹¹ of the northern Mid-Atlantic Ridge (R, I-IV¹¹; E, I, J¹⁰). A vertical bar indicates variations where the topography is too irregular to determine a unique depth. The dashed line is part of a sine curve varying from maximum elevation (zero depth) at the latitude of the North Atlantic pole of rotation (62° N, ref. 1) to minimum elevation (maximum depth) at the equator of rotation, 90° to the south.

topographic slope with increasing spreading rates is a result of the greater length of layer 2 crust generated per unit time, as well as an almost linear decrease in the absolute elevation of the crest of the ridge associated with the thinning of layer 2.

From the lithospheric plate motions¹⁻³ and the relationships between spreading rate and sea floor topography⁷⁻⁹, it follows that the depth of a mid-ocean ridge crest increases from a maximum elevation (zero reference depth) near the pole of rotation with the sine of the distance from the pole, and the slope of its adjacent flanks decreases with the reciprocal of the sine of the distance from the pole of rotation of the two lithospheric plates the boundaries of which form the ridge. The limiting case of zero slope and zero elevation (maximum depth) along the equator of rotation never occurs because it would require an infinite rate of sea floor spreading.

Sufficient data exist to test this hypothesis over about 40° of latitude in the North Atlantic. The depths of the Mid-Atlantic Ridge, measured at the inner margin of the High Fractured Plateau province, decrease between 49° and 12° N and this decrease may be approximated by a sine curve originating at the latitude of the pertinent North Atlantic pole of rotation (62° N) (Fig. 1, and see ref. 1). The variation in depths about the sine curve is a result of the irregular topo-

graphy of the ridge crest transected by numerous fracture zones. The southward decrease in crestal elevation and slope of the ridge flanks is apparent in profiles perpendicular to the ridge axis (Fig. 2). To measure depth and slope, the irregular topography of the High Fractured Plateau and Flank provinces was fitted with straight line segments through the mean topographical values. The depth of both provinces progressively increases and the slope decreases with distance from the pole of rotation. A marked topographic step between the High Fractured Plateau and the Flank provinces is evidence of a discontinuity in sea floor spreading, when the ridge subsided during a spreading lapse ending about 10⁷ yr ago¹⁰ (a discontinuity of zero spreading would result in a vertical topographic step of infinite slope). The progressive southward increase in the width of the High Fractured Plateau province is consistent with the increase of spreading rates with distance from the pole of rotation.

The maximum depth along the axes of the basins on the east and west margins of the Mid-Atlantic Ridge increases away from the pole of rotation, measured between 55° N and 12° N (Fig. 1). The axes of maximum depth can be approximated by sine functions, with the exception of an anomalous decrease in depth along the part of the western basin adjacent to the Lesser Antilles island arc, where special structural conditions prevail. The increase in depth at the axes of the marginal basins is less than that at the ridge crest, so that their respective sine curves converge towards the equator of rotation.

The observation that the increase in depth at the ridge crest and at the axes of the marginal basins can both be described by sine functions, originating near a pole of rotation and converging towards the equator of rotation, indicates that depth distribution is maintained as the sea floor spreads bilaterally away from the crestal region and subsides on the ridge flanks. The hypothesis presented here helps to explain the correspondence between depth and latitude in the oceans, because the poles of lithospheric plate rotation tend to lie near the Earth's rotational axis.

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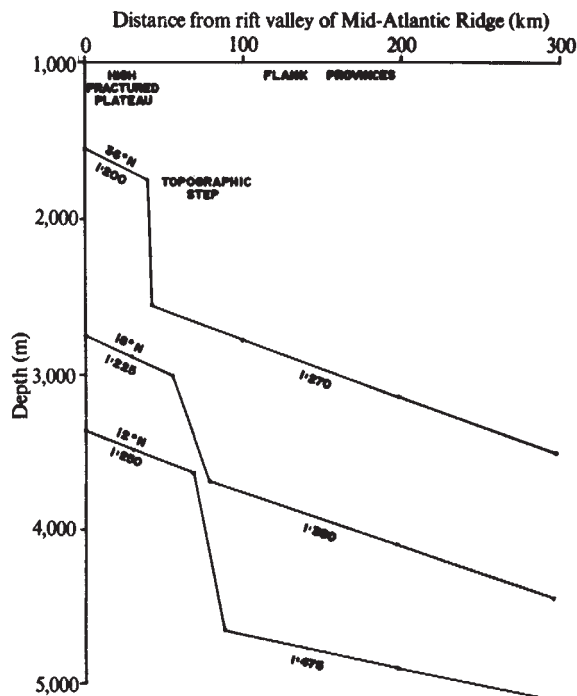


Fig. 2 Half-profiles perpendicular to the crest of the Mid-Atlantic Ridge. The High Fractured Plateau and Flank provinces are represented by straight line segments fitted through the mean topographical values with slopes as indicated¹⁰. The topographic step between provinces is evidence of a lapse in sea floor spreading which occurred simultaneously ending about 10⁷ yr ago¹⁰.

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BIOLOGICAL SCIENCES

Blood Concentration of Noradrenaline in the Dog after Intravenous Administration and the Effects of Desipramine

A RAPID intravenous injection leads to a "slug" of relatively undispersed drug traversing the arteries on the first circuit. Thereafter, due to differences in circuit times through different pathways, the drug will be dispersed and the "slug" will acquire a "tail"^{1,2}. When the drug is removed or inactivated by tissues, the tail will be at a much lower concentration than would be expected merely from dispersion of the drug into a larger volume. For example, after an intravenous dose of noradrenaline, up to 90% disappears in one passage through peripheral vascular beds³⁻⁶, so that the amounts left for dispersal in the subsequent circuits are small. Drugs which interfere with the removal process in peripheral vascular beds might therefore significantly increase the amounts of noradrenaline recirculating.

We have injected noradrenaline intravenously into dogs and measured the peak arterial concentration during the first circuit and the concentration in the "tail". We have also determined the effects of desipramine on these blood concentrations of noradrenaline.

Dogs of either sex weighing 8-14 kg were anaesthetized with sodium pentobarbitone (30 mg/kg given intravenously); anaesthesia was maintained by subcutaneous injections as required. An endotracheal tube was inserted and when necessary the lungs were ventilated mechanically with a Palmer respiration pump. Polyethylene catheters were tied into the right femoral artery for removal and into the right femoral vein for replacement of blood. Mean arterial pressure was recorded on a Beckman Offner dynograph by a Statham transducer attached to a catheter in the right femoral artery. Intravenous injections were made through a catheter in the left femoral vein. Noradrenaline in the blood was estimated by the blood-bathed organ technique^{7,8} by pumping arterial blood at a rate of 10 ml./min continuously over rat stomach strips⁴; blood returned to the dog through a femoral vein. The movements of the stomach strips (loaded with 2 g) were detected with auxotonic levers⁹ and Harvard transducers and recorded on the dynograph. The dog was given heparin intravenously (10 mg/kg) before the blood was superfused over the assay tissues; in some experiments an additional 5 mg/kg was injected 5-6 h later.

To assess the peak circulating concentration of noradrenaline after intravenous injections its effects on the stomach strips were matched or bracketed by injections of noradrenaline made directly into the superfusing blood (IBB injections). Dose-response curves were plotted from the effects of the IBB injections on the rat stomach strips, both before and after treatment of the dog with desipramine. Thus any changes in the sensitivity of the assay tissues were noted. In three of the twelve experiments, there was an increased sensitivity of the rat stomach strips to noradrenaline after desipramine. But the increase in sensitivity applied to IBB injections as well as to the intravenous ones, and so the assay itself was not affected.

In a typical experiment (Fig. 1) the degree of relaxation of the rat stomach strip induced by the intravenous injection of 5 µg noradrenaline was intermediate between the relaxations induced by 25 and 50 ng noradrenaline injected directly into the superfusing blood. The dog was then given desipramine (3 mg/kg, intravenously) and 30 min later the noradrenaline injections were repeated; the degree of relaxation of the rat stomach strip to intravenous noradrenaline (5 µg) was still between those induced by 25 and 50 ng injected directly into the superfusing blood.

This lack of increase in the peak concentration of circulating noradrenaline after desipramine was found in eight of twelve experiments. In the other four the peak concentration of noradrenaline was increased by 15-50% after treatment with desipramine. Such changes in peak concentration during the first circuit would be expected only if a significant removal or inactivation of the intravenous dose of noradrenaline had occurred in the blood, heart chambers, or lungs, and if this inactivation were inhibited by desipramine. Ginn and Vane⁵ showed that up to 30% of an intravenous infusion of noradrenaline in the dog disappeared in a single passage through the pulmonary circulation.

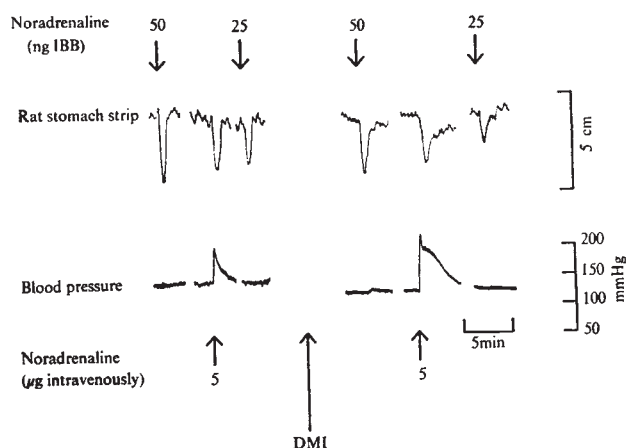


Fig. 1 A rat stomach strip (top trace) was superfused with blood from an anaesthetized dog. An intravenous injection of noradrenaline gave a rise in blood pressure (lower trace). The peak concentration of noradrenaline in the circulating blood, as shown by the relaxation of the rat stomach strip, was between the concentrations elicited by injections of noradrenaline of 25 and 50 ng given directly (IBB) to the assay tissue. After desmethylimipramine (DMI, 3 mg/kg given intravenously), the pressor effect of noradrenaline was potentiated, but the peak concentration in the circulating blood did not change. Note, however, the more prolonged relaxation of the rat stomach strip after the intravenous injection. Time 5 min; vertical scales, 5 cm and mm Hg.

To estimate noradrenaline in the blood after the passage of the peak concentration, the roller pump, which ordinarily supplied blood to the assay tissues from the arterial catheter, was connected to a syringe containing arterial blood drawn from the dog immediately before injection. Blood from the syringe was supplied for the first 30 s after intravenous administration of the noradrenaline. Thus the assay tissues were not exposed to the initial peak of noradrenaline but only to that which was circulating after 30 s (circulation time in the dog 10-20 s, ref. 10). The relaxations of the rat stomach strip induced by the noradrenaline remaining in the blood after 30 s were matched or bracketed by relaxations induced by IBB infusions of noradrenaline (Fig. 2). The noradrenaline in blood 30 s after an intravenous injection of 0.5 µg/kg caused a relaxation of the rat stomach strip which was less than that evoked by a 2 min infusion of noradrenaline directly into the superfusing blood to give a concentration of 1 ng/ml. After intravenous injection of desipramine (3 mg/kg) the noradrenaline remaining in the blood 30 s after the intravenous dose of 0.5 µg/kg caused a relaxation of the rat stomach strip which was greater than before. The effect was now matched by an infusion of noradrenaline directly into the superfusing blood to give a concentration of 4 ng/ml. for the first minute, 2 ng/ml. for the second minute and 1 ng/ml. for the third minute. The results illustrated in Fig. 2 and from six similar experiments suggest that the concentrations of recirculating noradrenaline are increased by treatment with desipramine, although it is possible that the injected noradrenaline caused

the release of some other rat stomach strip-relaxing substance into the circulation. A large increase in the pressor effects of noradrenaline on the blood pressure after desipramine (as in Figs. 1 and 2) was consistently seen in all these experiments.

Noradrenaline in the blood was also estimated in dog hind-leg perfusion experiments. Arterial blood was supplied to a constant-flow (30–40 ml./min) Sigmamotor pump from a cannula in the lower aorta and delivered to the leg through a cannula in the femoral artery. The dog was anaesthetized with pentobarbitone and heparin was used as an anticoagulant. The femoral and sciatic nerves were cut, and mass ligatures which excluded the femoral artery and vein were tied around the leg. Intra-arterial injections were made into a thick-walled rubber tube connected to the femoral arterial cannula. Intravenous injections were made into a polyethylene cannula inserted into the femoral vein of the opposite leg.

Desipramine (3 mg/kg, given intravenously) potentiated the rise in both systemic blood pressure and hind-leg perfusion pressure produced by noradrenaline (Fig. 3). The small potentiation of the effects on perfusion pressure of an intra-arterial injection of noradrenaline could have been due to a change in receptor sensitivity or to an inhibition of local uptake mechanisms in the vessel walls, allowing more noradrenaline to reach the receptor. However, the fact that the rise in perfusion pressure of the leg induced by intravenous injection of noradrenaline was potentiated to a much greater extent must mean that such local changes did not contribute substantially to the potentiation. Desipramine must therefore have increased the amounts of noradrenaline reaching the hind leg after an intravenous injection.

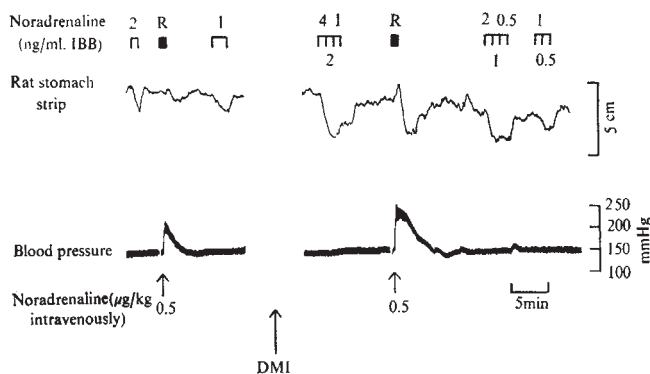


Fig. 2 A rat stomach strip (top trace) was superfused with blood from an anaesthetized dog. During the first 30 s after an intravenous injection of noradrenaline (0.5 µg/kg) the strip was superfused with blood from a reservoir (R), as described in the text. The concentration of noradrenaline in the blood after 30 s was less than 1 ng/ml., as shown by the relaxation of the rat stomach strip. After desmethylimipramine (DMI, 3 mg/kg given intravenously), the noradrenaline injection was repeated. The blood after the first 30 s now contained noradrenaline at 4 ng/ml. Time 5 min; vertical scales, 5 cm and mm Hg.

These results distinguish two factors which contribute to the potentiation of the blood pressure response to noradrenaline which is seen after desipramine.

First, an occasional change of not more than 50% in the peak concentration of the "slug" of injected noradrenaline during the first circuit, presumably due to a reduction of uptake in the lungs. Second, a change in the concentration of the noradrenaline which recirculates as a "tail". This may be increased three or four-fold; the duration of the tail is also increased, so that blood concentrations of noradrenaline of 1 ng/ml, which were previously only detected 30 s after injection were, after desipramine, detected up to 3 min after injection of noradrenaline.

In addition to these increases in the concentration of circulating noradrenaline, other factors can contribute to the

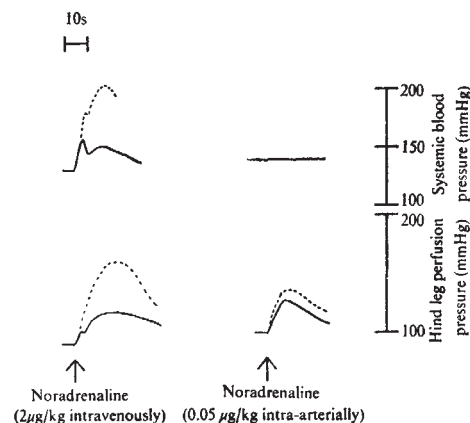


Fig. 3 The hind leg of a dog was perfused with blood, as described in the text. The top trace is blood pressure and the lower one perfusion pressure. The solid lines show the effects of noradrenaline before and the dotted lines the effects after desmethylimipramine (3 mg/kg given intravenously). The effects on the perfusion pressure of intravenous noradrenaline were potentiated much more than the effects of intra-arterial noradrenaline (0.05 µg/kg) given directly to the perfused leg. Time 10 s, vertical scales in mm Hg.

potentiation. Sigg, Soffer and Gyermek¹¹ reported a peripheral sensitization of adrenoceptors by imipramine and desipramine. There might also be a local increase in the concentration of noradrenaline reaching the receptors, because of inhibition of uptake into nearby uptake sites, for both imipramine and desipramine block catecholamine uptake, especially into tissues rich in adrenergic nerve endings¹². Our results, however, suggest that the contribution to the potentiation from either of these mechanisms is small, because of the relative lack of potentiation of the effects of intra-arterial noradrenaline on the perfused hind leg. The blood-bathed organs also showed little or no potentiation to the effects of noradrenaline after desipramine. Sigg, Osborne and Korol¹³ and Eble¹⁴ have shown that imipramine blocks cardiovascular homeostatic reflexes. Such an effect would allow a much greater blood pressure response to intravenous noradrenaline, but would not account for the substantial increase in the effects of intravenous noradrenaline on the perfused denervated hind leg.

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Antigen-binding Small Lymphocytes in Delayed Hypersensitivity to Tuberculin

Small lymphocytes are known to act as specific mediators of delayed hypersensitivity reactions and immunoglobulin (Ig) determinants^{1,2} and antigen-specific receptor sites^{3,4} have recently been demonstrated on the lymphocyte surface. We have investigated in guinea-pigs the numbers of circulating small lymphocytes binding tuberculin in relation to the development of delayed hypersensitivity to tuberculin during primary immunization with BCG vaccine.

Groups of four to six guinea-pigs were killed at intervals up to 28 days after primary immunization with 0.1 ml. of BCG (Glaxo) in Freund's incomplete adjuvant (Difco) into each footpad. Half of each group of animals was skin-tested with 10 µg of purified protein derivative of mammalian tuberculin (mammalian PPD; Central Veterinary Laboratories, Weybridge), and 24 h later the size of the delayed hypersensitivity skin reactions was measured immediately before the animal was killed. Leucocyte-rich plasma was obtained by dextran sedimentation of cardiac blood: after centrifugation, the leucocytes were resuspended in TC 199 (Glaxo). ¹²⁵I-mammalian PPD (specific activity 140 µCi/µg), prepared by the method of Hunter and Greenwood⁵, was added to 1.6 ml. of leucocyte suspension: as controls, leucocytes were mixed with unlabelled mammalian PPD and ¹²⁵I-human growth hormone. The final concentration of antigen in each case was 10 ng/ml. The leucocyte-antigen mixtures were incubated at 37° C for 45 min, then washed in TC 199 and the residual erythrocytes were lysed by osmotic shock. Smears of the final centrifuged deposits of leucocytes were fixed in absolute methanol, dipped in Ilford K2 nuclear emulsion, developed after 2 weeks of exposure and stained by Leishman's method.

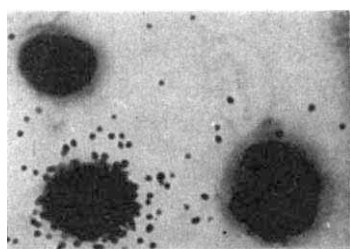


Fig. 1 Autoradiograph showing an ¹²⁵I-PPD binding lymphocyte (bottom left) and unlabelled lymphocyte (top left), polymorphonuclear leucocyte (bottom right) and an erythrocyte ghost (top right). (Leishman stain, ×1,000.)

We have detected binding of ¹²⁵I-mammalian PPD by circulating cells which looked like small lymphocytes (Fig. 1) in the light-microscope. The lymphocytes that were covered by more than twenty silver grains were considered positive: the concentration of silver grains on most of the remaining lymphocytes was little greater than background. The percentage of antigen-binding lymphocytes was determined by counting approximately 1,000 small lymphocytes in each smear; the slides were coded and the pattern of movement during oil immersion examination was standardized to avoid bias.

Fig. 2 shows the average percentage of circulating ¹²⁵I-PPD binding small lymphocytes at intervals throughout the 28 day experimental period. In the group which had not been skin tested, values rose from a base-line of 0.4% before immunization to a maximum of 14.2% on the fifth day and thereafter fell to 1.5% by the twenty-first day. The animals in the skin tested group behaved similarly up to the fifth day, but from the tenth to the twenty-eighth day substantially more small lymphocytes bound labelled PPD in this group probably because of a secondary response provoked by the PPD skin test. During the

experimental period between 0.2% and 0.6% of small lymphocytes bound ¹²⁵I-human growth hormone; a small rise coincided with the peak of ¹²⁵I-PPD binding lymphocytes.

The delayed hypersensitivity (DH) skin reaction to mammalian PPD was first observed on the fifth day after immunization. The size of the DH skin reaction, estimated semi-quantitatively by the index (radius² × thickness), increased progressively to the twenty-eighth day (Fig. 2). DH skin reactions to mammalian PPD became progressively more intense after the peak of circulating ¹²⁵I-PPD binding small lymphocytes.

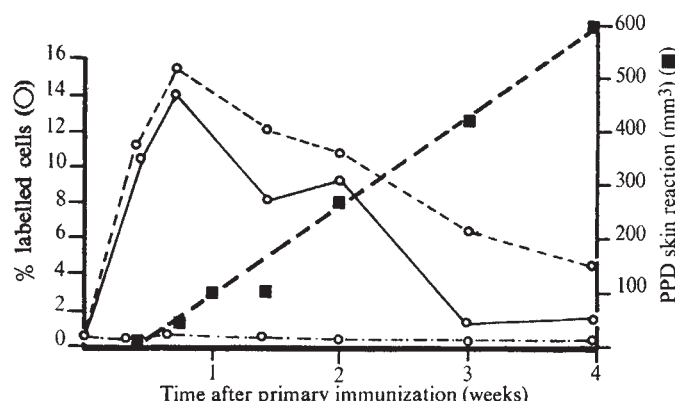


Fig. 2 ¹²⁵I-PPD binding by peripheral blood lymphocytes (---, animals skin tested with PPD 24 h earlier; —, animals not skin tested) and semi-quantitative estimate of delayed hypersensitivity skin reaction to PPD in guinea-pigs immunized with BCG. - - - - - , ¹²⁵I-HGH.

The immunological specificity of labelled PPD binding by small lymphocytes was characterized by blocking experiments. Leucocytes from six guinea-pigs, immunized 10 days earlier, were pre-exposed to 600 ng/ml. of unlabelled antigens (Table 1). Pre-exposure to unlabelled mammalian PPD reduced the percentage of ¹²⁵I-mammalian PPD binding small lymphocytes from 10.1% to 0.4%: pre-exposure to avian PPD and 'Johnin' (Central Veterinary Laboratories), both of which cross-reacted in DH skin reactions, similarly reduced the percentage of ¹²⁵I-mammalian PPD binding small lymphocytes to 0.9%. In contrast, substantially greater percentages of small lymphocytes were found to bind labelled mammalian PPD after pre-exposure to the unrelated antigens, mallein (Central Veterinary Laboratories), aspergillosis antigen No. 1 (Bencard) and human growth hormone (MRC standard preparation): we believe that this much less marked interference with antigen binding is not a specific immunological phenomenon.

Table 1 Blocking of Labelled PPD Binding by Peripheral Blood Lymphocytes of Guinea-pigs 10 Days after Primary Immunization with BCG

Cells pre-exposed to TC 199 containing	Cells labelled with ¹²⁵ I-mammalian PPD (%)	
	Mean	s.d.
No antigen	10.1	2.0
Mammalian PPD	0.4	0.4
Avian PPD	0.9	0.6
'Johnin'	0.9	0.5
Mallein	4.7	2.5
Aspergillosis antigen No. 1	6.6	2.4
Human growth hormone	6.6	3.0

The effect of masking Ig-type determinants on the surface of small lymphocytes with appropriate antisera was studied with six guinea-pigs 10 days after primary immunization. The number of small lymphocytes that bound labelled mammalian

Table 2 Inhibition of Labelled PPD Binding by Peripheral Blood Lymphocytes of Guinea-pigs 10 Days after Primary Immunization with BCG

Cells pre-exposed to rabbit serum Source	Antibody	Cells labelled with ¹²⁵ I-mammalian PPD (%)	
		Mean	s.d.
Behringwerke AG	Anti-G-P γ -globulin	2.1	1.9
Nordic diagnostics	Anti-G-P Ig	1.2	0.9
Aberdeen University	Non-immune	7.0	3.3

PPD was considerably reduced by pre-exposure to 1:8 dilutions of two commercially prepared rabbit anti-guinea-pig Ig sera, but not by pre-exposure to non-immune rabbit serum (Table 2).

Circulating and lymph node lymphocytes from immune animals have been shown to bind the corresponding radioisotope-labelled antigen^{4,6,7}, but the only evidence for the specificity of this reaction was blocking by pretreatment with unlabelled homologous antigen⁶ or by masking of Ig-type determinants on the cell surface^{6,7}. To establish the immunological specificity it is important to compare the effects of different antigens in absorption experiments: we have now shown that the homologous and two closely related antigens block this reaction almost completely, whereas there was limited, apparently non-specific, interference by heterologous antigens. The differential effects on absorption, the inhibition by masking of Ig-type determinants and the apparent secondary response after skin testing are, we believe, strong evidence for the immunological specificity of labelled antigen binding by circulating lymphocytes in our experiments.

Sensitized small lymphocytes are believed to be the specific mediators of DH skin reactions, and so it is interesting that we have found that circulating antigen-binding small lymphocytes were most numerous 5 days after primary immunization when DH skin reactions were hardly detectable. Although the antigen-binding small lymphocytes which appear early after primary immunization may not be the potential effector cells of DH skin reactions, they may later mature into specific mediator cells, or they may require to cooperate with factors, such as other lymphoid cells, or macrophages or humoral antibody, in order to effect the DH response.

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Chromosomal Evidence for Natural Interspecific Hybridization by Mosquitoes of the *Anopheles gambiae* Complex

Mosquitoes of the *Anopheles gambiae* complex are a closely related group of species that cannot all be distinguished easily on usual morphological grounds^{1,2}. The complex consists of

five known sibling species in Africa^{3,4} of which *A. melas* Theobald and *A. merus* Donitz breed in salt water, and forms provisionally known as species *A*, species *B* and species *C* breed in freshwater. Species *C* is not markedly anthropophilic^{1,5}, but species *A* and *B* are the primary vectors of human malaria throughout much of Africa, and *A. merus* and *A. melas* are malaria vectors of lesser importance in the coastal regions of East and West Africa^{1,5}. Individuals of the species which breeds in freshwater can only be identified with certainty by the banding patterns of their polytene chromosomes⁷⁻¹⁰.



Fig. 1 Species *A*/species *B* asynaptic polytene *X*-chromosomes from an ovarian nurse cell nucleus of a wild-caught hybrid female mosquito of the *Anopheles gambiae* complex. From left to right the four visible ends are: centromere attachment of *A*, centromere attachment of *B*, free end of *B*, free end of *A*. The characteristic differences between species *A* and species *B* chromosomes are to be seen at the free ends.

The designation of these forms as separate species has depended on two factors. First the five forms of *A. gambiae sensu lato*, though they readily hybridize in laboratory cages or when force-mated, do not give fully fertile hybrid offspring^{1,3}. All the twenty possible crosses can yield hybrid adults, but the heterogamic males produced are always virtually sterile with underdeveloped testes. Female progeny from most crosses have slightly reduced fertility when back-crossed with parental males, and some crosses produce no females or a predominance of sterile males^{1,4}.

Second, sterile males do not normally occur in places where two or more of the forms of *A. gambiae sensu lato* coexist¹¹⁻¹⁴. This indicates that assortative matings are the rule in nature, which provides justification for regarding these forms as distinct species¹⁵, whatever their genetical compatibility when induced to copulate artificially.

There are four records of apparent interspecific hybridization in nature between sympatric wild populations of separate species within the *A. gambiae* complex. Two of these reports are of uncertain validity because they involved the collection of wild forms morphologically intermediate between *A. melas* and species *A* populations^{16,17}. The third report concerns the production of sterile male progeny by twelve out of thirty-eight females collected at Houndé in Upper Volta where species *A* and *B* coexist¹⁷. Additional sterile males could not be found subsequently in this locality¹. The fourth report comes from a survey at Jericho, Ibadan, Nigeria, where some probable species *B* and most species *A* were shown to occur sympatrically¹². One brood containing all sterile males was found among 202 families obtained from wild *A. gambiae sensu lato* females. Elsewhere, workers have searched in vain for sterile males in places where two¹¹⁻¹³ or three¹⁴ species of the complex occur together.

I present here cytogenetic proof of four cases of natural hybridization between species *A* and species *B* in East Africa. The hybrids—females captured indoors at the village of Seger, near Korogwe, in Tanzania during February to May 1970—were recognized by the complete asynapsis of polytene maternal and paternal *X*-chromosomes in nuclei of ovarian nurse cells^{1,7}. Each of the asynaptic *X*-chromosomes in each nucleus displayed distinct banding patterns recognizable as those characteristic of species *A* and *B*⁸ (Fig. 1).

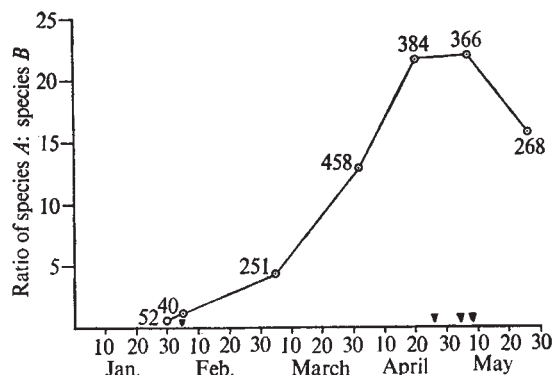


Fig. 2 Changes in the ratio of species *A* : species *B* among female mosquitoes of the *Anopheles gambiae* complex resting indoors at Seger, Tanzania, during February to May 1970. The numbers of females examined through a series of working days are shown beside each point on the graph. ▼, The four dates on which hybrid species *A* × species *B* females were captured.

During the early part of 1970, mosquitoes of the *A. gambiae* complex were collected regularly from indoor resting places at Seger, and the females were specifically identified by Coluzzi's cytogenetic method⁹. From 1,830 identifications made during January to May 1970, 90% were species *A* and 10% were species *B*. The four hybrids represented 0.22% of all the females identified.

A rainy season commenced in mid-February at Seger, bringing about a great increase in the numbers of species *A* but not species *B*. The ratio of *A* to *B* rose from 0.6 in January to 21.6 in late April, 21.9 in early May and then decreased to 15.8 by late May (Fig. 2). Hybrid females were captured on February 5, April 27 and May 4 and 6 (Fig. 2), but not during mid-February to mid-April during which more than 700 cytotoxic identifications were made. Thus, hybridization was first detected when species *A* populations surpassed the number of species *B*, and occurred again when the increased proportion of species *A* stabilized at 96% of all *A. gambiae sensu lato* females resting indoors.

Complete results of this continuing study will be published elsewhere, but the data given here reveal that considerable fluctuations occur independently in the densities of species *A* and species *B* of *A. gambiae* apparently causing a partial breakdown in the mating barrier in certain, so far undefined, ecological circumstances. Interspecific hybridization seems to be rare at Seger, probably because the reproductive behaviour patterns of species *A* and *B* are dissimilar. But the fact that wild, and presumably fertile, hybrid *A* × *B* females occur suggests that genetic material is occasionally transferred between these two cryptic species through the mechanism of introgressive hybridization^{18,19}.

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Molecular Mechanisms of Storage of Transmitters in Synaptic Terminals

MUCH more is known about the molecular constitution of the storage mechanism for catecholamines than about how acetylcholine (ACh) is stored, although morphine and related drugs are known to inhibit its release. Because adenosine triphosphate (ATP) seems to be involved in the storage of catecholamines in the chromaffin granules of the adrenal medulla we looked for a special affinity between morphine and various phosphorylated nucleosides which might be concerned in the storage of ACh. Acetylcholine itself could not be tested by these techniques because it lacks native fluorescence.

We measured the reduction of native fluorescence of morphine sulphate when added to successive amounts of the various nucleotides as described previously¹. Apomorphine (HCl), d-LSD (tartrate) and tyramine (HCl) were used as controls. The nucleotides used were adenosine monophosphate (AMP), cytosine monophosphate (CMP), inosine monophosphate (IMP), and uridine monophosphate (UMP); CMP and UMP dinucleotides (CC and UU); (sodium salts); and poly A, poly C, poly I and poly U (potassium salts).

Figs. 1–3 show the results for morphine, apomorphine and d-LSD respectively. Both morphine and apomorphine bind best to CMP and to poly C. In the case of morphine the curves for poly C and poly IC are practically identical. This suggests that morphine is binding on some locus in poly C that is not masked when the IC bonds are formed, whereas this is not the case for apomorphine. d-LSD has quite a different pattern, binding best to poly U. Morphine also bound better to CC than UU; apomorphine hardly bound to UU at all, and with CC the native fluorescence increased. Tyramine bound well to all four mononucleotides.

To explain these results we must take into consideration the nature of the secondary conformation of these nucleotides. At the temperature, pH and salinity used in these experiments poly C stacks well and possesses lengths of single helix, poly U does not stack at all and is a random coil, poly A is intermediate between these and the conformation of poly I is not known. The order of binding of morphine is poly C > poly A > poly U, which suggests that good stacking promotes binding. In the case of the mononucleotides CMP is still the best, but

the order of A and U reverses. Apomorphine and d-LSD hardly bind to the mononucleotides. Stacking is promoted by decreasing the temperature, pH and salinity. These factors were tested by comparing the binding of morphine, d-LSD

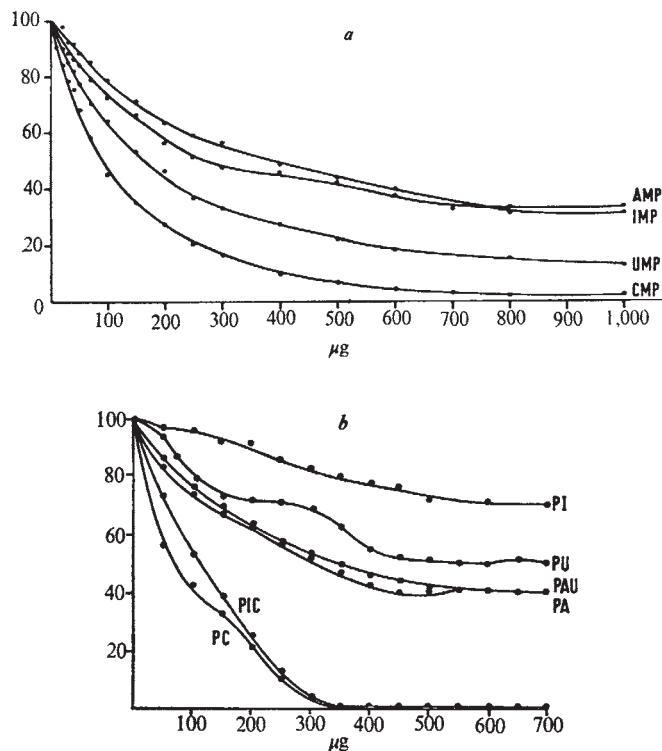


Fig. 1 The reduction of native fluorescence of 10 µg/ml. of morphine sulphate (290 : 345) on adding the nucleotides indicated in water expressed as a percentage of the original reading. Abscissa amount of nucleotide in µg/ml. (a) Mononucleotides; (b) polynucleotides.

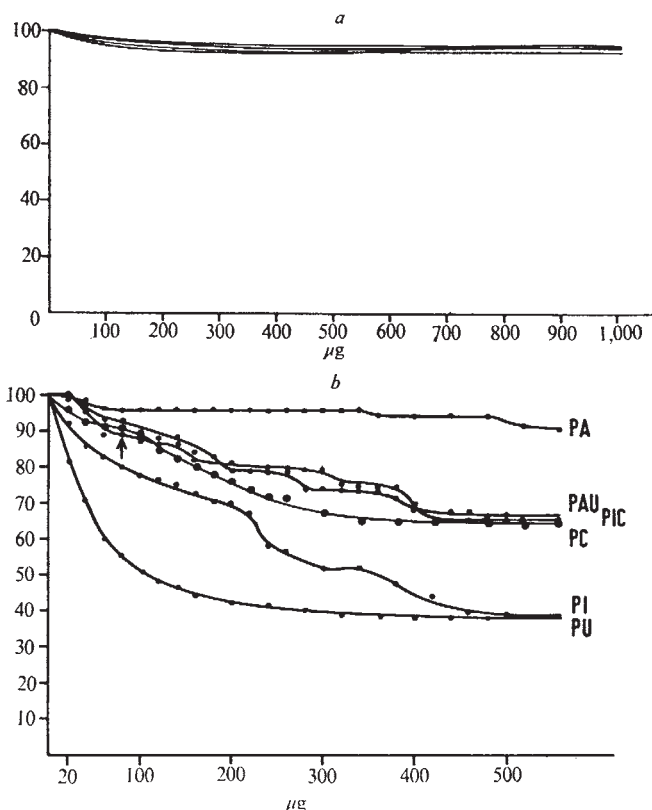


Fig. 3 As Fig. 1 for d-LSD (tartrate; 1 µg; 330 : 430).

and tyramine to poly C at (a) pH 7.5, normal saline and 15° C and (b) pH 3.0, water and 4° C. These results (Table 1) show that the binding in (b) is better in all cases.

Table 1 Binding Properties of Tyramine, d-LSD and Morphine

Drug	Poly C	Reduction of fluorescence (%)	
		(a)	(b)
Tyramine (1 µg)	40 µg	18	70
d-LSD (1 µg)	80 µg	8	40
Morphine (10 µg)	200 µg	74	82

The binding of tyramine, d-LSD and morphine to poly C (a) pH 7.5, normal saline and 15° C; (b) at pH 3.0, water and 4° C.

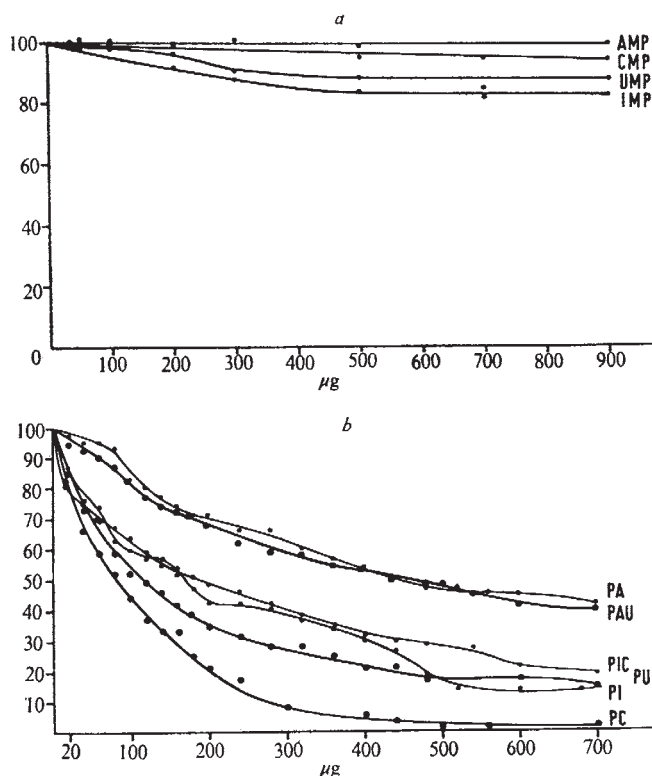


Fig. 2 As Fig. 1 for apomorphine (HCl; 1 µg; 320 : 340).

We tested for any possible physical quenching by CMP of morphine because CMP absorbs at a maximum of 280 nm as against 290 nm for morphine. So we activated 500 µg of CMP at 290 nm and recorded the fluorescence scan. The molecule, however, only fluoresced very feebly (1/200 morphine) at this wavelength suggesting that its absorption of photons was low. Finally we fluoresced 10 µg/ml. morphine as before, but first passed the incident light through a solution with 500 µg of CMP in a similar quartz container with a water control. This resulted in no reduction of morphine fluorescence. Thus the effect observed in these experiments is unlikely to be due to physical quenching.

CPK molecular models were then studied to see how ACh might bind to a stacked array of CMP molecules, and how morphine could also bind and inhibit release. The fact that morphine binds equally well to poly IC as to poly C suggests that the polar groups on cytosine are not involved. Morphine has a highly lipophilic molecule, and the even more potent derivative shown in Fig. 4 is even more lipophilic, so it would be unlikely to bind to the highly hydrophilic phosphate groups. The most likely mode of primary binding of ACh would be

an ionic bond from the onium head to one of the phosphate oxygens. If this bond is made, we observed that the geometry of the stacked CMP molecules provides two accessory binding sites for ACh (if the CMP molecules are arranged in a single helix so that the $(\delta-)$ O and the $(\delta-)$ 3N of one are in close contact with the $(\delta+)$ 2C and $(\delta+)$ 4C of its neighbour). The ether oxygen of ACh can bind to the phosphate hydroxyl and this locates the methyl group of ACh in lipophilic contact with the adjacent ribose 3 CH and 4 CH groups (Fig. 5). Fig. 5 also shows how the C-ring variant of morphine (Fig. 4), which is considerably more potent than morphine, now fits closely into this complex. Fig. 4b shows the appearance of this molecule looking down at the lipophilic surface of the B and C rings. This surface presents a protonated nitrogen in the middle of the upper part, which is surrounded by a lipophilic ring. This surface is complementary in its size, shape, charge and lipophilic distribution to the surface of the helically packed CMP molecules (on the side corresponding to the major groove of RNA). The complementary negativity is provided by one spare orbital on the ribose ring oxygen and the lipophilic ring is provided by the 1 CH, 4 CH and 5 CH groups of ribose, and the entire 5 and 6 CHs of two adjacent cytosines. Lipophilic contact is also made with portions of the adjacent ACh and morphine molecules. The benzene ring now sticks vertically up in the air (for the molecule is essentially T-shaped) and its hydroxyl group can now make a hydrogen bond with the carbonyl group of ACh.

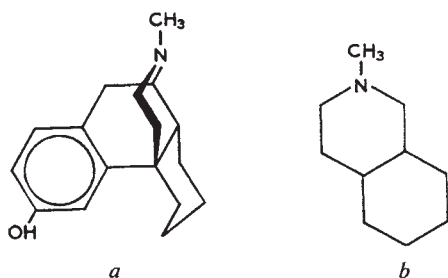


Fig. 4 (a) The C-ring variant of morphine; (b) side view.

We investigated whether ACh would increase the binding of morphine to poly C and CMP but it would not, perhaps because the morphine binding is optimum anyway. The necessary experiment (using some other techniques such as polarography) is to investigate whether morphine promotes or stabilizes the binding of ACh to poly C or CMP. Clouet and Williams² have shown that ³H-dihydromorphine binds strongly to RNA in brain.

Thus we would suggest, as a working hypothesis for further experiments, that a storage site for ACh consists of a stacked array of CMP molecules stabilized by divalent ions. The hypothesis may be tested by measuring the CMP content of ACh synaptosomes. This CMP-ACh complex need not be in the long term store inside vesicles but in the cytoplasmic "second pool" or possibly, as it is defined purely by the action of morphine, it may form part of a release or transport mechanism. The CMP-ACh complex bears close stereochemical relations to other narcotics such as methadone and meperidine. A study of a number of these indicates that the more potent the drug (for example, the Diels-Alder adduct of thebaine) the closer the relationship.

Next we considered catecholamines. The chromaffin granules of the adrenal medulla contain large amounts of ATP, Ca^{2+} , Mg^{2+} and protein, and can store four molecules of catecholamines or two molecules of serotonin per molecule of ATP³. The amines may be released by ACh, in which case prostaglandin $\text{F}_{1\alpha}$, and no other prostaglandin, is released⁴, together with the other contents. But the prostaglandin is probably located in the membrane of the chromaffin cell, for

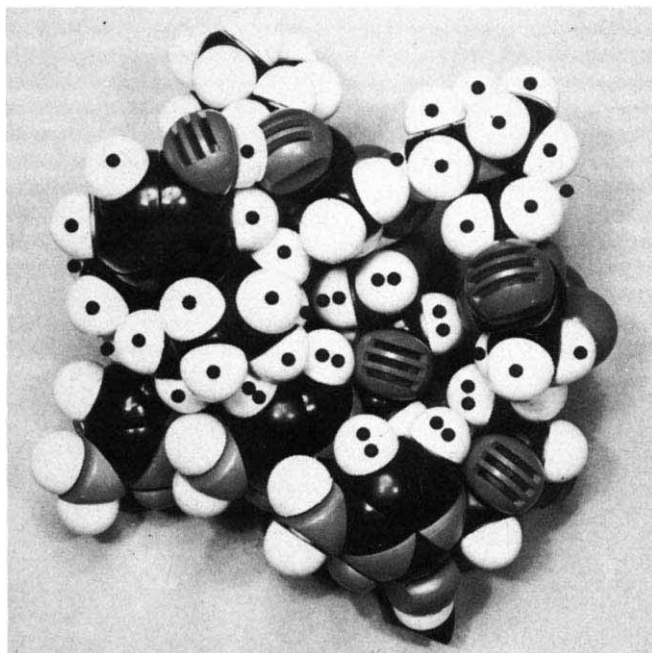


Fig. 5 CPK model of CMP units stacked in a helix binding ACh (single dots R.; also no dots L.) and the C-ring variant of morphine (single dots L.) in the manner indicated in the text. One "morphine site" is vacant of which the lipophilic ring Hs carry two dots.

there is no prostaglandin in the granule. ATP molecules are released unhydrolysed. It is possible that the macromolecular complex storing the amines is composed of these compounds and we wish to discuss how they could fit together in accordance with these facts. Abood and Matsubara⁵ have shown that ATP binds strongly to rat brain synaptosomal protein, probably by electrostatic binding to glutamine or asparagine moieties. Following up our suggestion that one storage site for ACh consists of stacked CMP molecules we considered whether a similar complex of ATP or of ATP attached to protein would have the required properties.

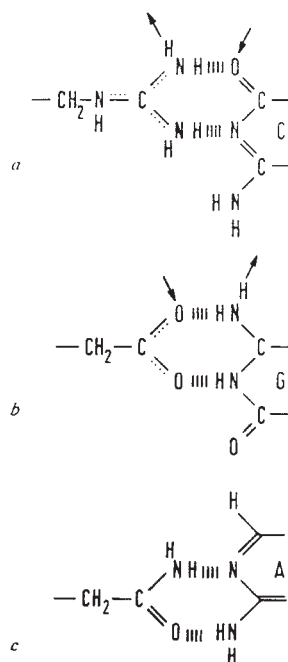


Fig. 6 The stereochemical relations between (a) cytidine and arginine, (b) guanine and glutamate, and (c) adenine and glutamine. The arrows indicate bond angles.

CPK models indicate that there is a possible way of constructing such a complex. Adenine is complementary to two amino-acids only—glutamine and asparagine (Fig. 6). A polypeptide chain in the β conformation, in which every other amino-acid is glutamine (or asparagine), can form a ladder-like complex with an array of ATP molecules, if the latter themselves are linked by two hydrogen bonds from the ribose 2 hydroxyl of one and the ribose 5 O of the next and between the 3 ribose of the first to the adjacent phosphate oxygen of the second, and by divalent ions linking the other phosphate oxygens. The intercalation site between the amino-acid-base pairs forming the rungs of the ladder is now the right thickness to admit a benzene or indole ring, and it could accommodate four molecules of a catecholamine or two molecules of serotonin (Fig. 7).

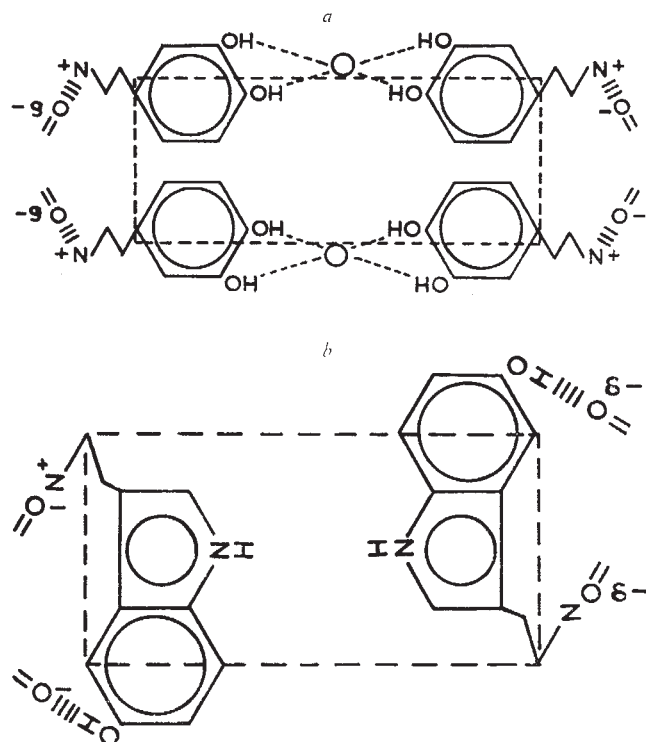


Fig. 7 A diagram of how the ATP-protein complex could bind (a) four molecules of a catecholamine (with chelation of a suitable metal ion) or (b) two molecules of serotonin. Alternatively, the phenolic OHs of one E can hydrogen bond to those of the other E.

The amino-groups on the intercalated amines can bind electrostatically to carbonyl Os and phosphate Os on each side. If every fourth or sixth amino-acid in this portion of chromogranin is Pro, a gentle helical formation results. This separates the phosphate Os, so fewer divalent ions would be needed to stabilize the complex.

We would therefore suggest that the storage site of catecholamines (and serotonin) in the adrenal medulla (and perhaps elsewhere) is a complex of ATP, a protein—probably chromogranin—which has segments in which every other amino-acid is glutamine (or asparagine), Ca^{2+} and Mg^{2+} constructed in the form of a ladder, in which the amines are stored by intercalation (and electrostatic binding) between the amino-acid-base pairs. Smith and Winkler⁶ have reported that 156 of the amino-acids obtained by the hydrolysis of the soluble protein of the adrenal chromaffin granules (chromogranin) are glutamate and fifty-six are aspartate, of which probably at least seventy are in the amide form. These may supply sufficient glutamine (asparagine) moieties for this hypothesis. Each molecule of chromogranin can bind ninety molecules of ATP. Helle⁷ found that ATP is firmly bound to

the protein. This hypothesis may be tested by observing the effect on amine storage of polypeptides with the repeating sequence Gln-Gly-Gln-Gly (or Pro)—(since Gln is complementary to itself), and by determining the amino-acid sequence of chromogranin. Because this molecule consists of two identical subunits, and in order to be able to bind four molecules of E per molecule of ATP by intercalation, all the glutamine (or asparagine) moieties in each subunit must be contiguous. Thus each subunit of chromogranin must have a ninety amino-acid segment in which every other amino-acid is glutamine or asparagine.

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Model for the Action of Tetrodotoxin and Batrachotoxin

It has been suggested¹⁻³ that the ionic permeability of membranes is controlled by complex formation on the surface between various phosphorylated nucleosides and protein chains. This is based on the fact that guanine, cytidine and adenine are complementary to glutamate, arginine, and glutamine respectively and all four bases are complementary to glutamine. Adenosine triphosphate (ATP) has been reported to bind strongly to rat brain synaptosomal protein—probably to glutamine moieties⁴. It is postulated that complex formation is promoted by prostaglandins and disrupted by transmitters and on this basis it has been possible to suggest working molecular specifications for acetylcholine (ACh), γ -aminobutyric acid (GABA) and glutamate receptors based on guanine-glutamate and cytidine-arginine pairs, and for prostaglandin and catecholamine action based largely on the adenine-glutamine pairs. This hypothesis has explained a quantity of structure-activity relationship data on agonists and antagonists of these compounds as detailed in refs. 1-3.

In this communication, we apply this concept to the Na^+ channel through axonal and muscle membrane (outside the chemosensitive end plate) blocked by tetrodotoxin (TTX) and kept forcibly open by batrachotoxin (BTX) which act uniquely here.

Investigations using Corey-Pauling-Kaltun molecular models indicate that the molecule of tetrodotoxin bears close stereochemical relationships to two strands—one of a polypeptide and the other to two molecules of a phosphorylated nucleoside.

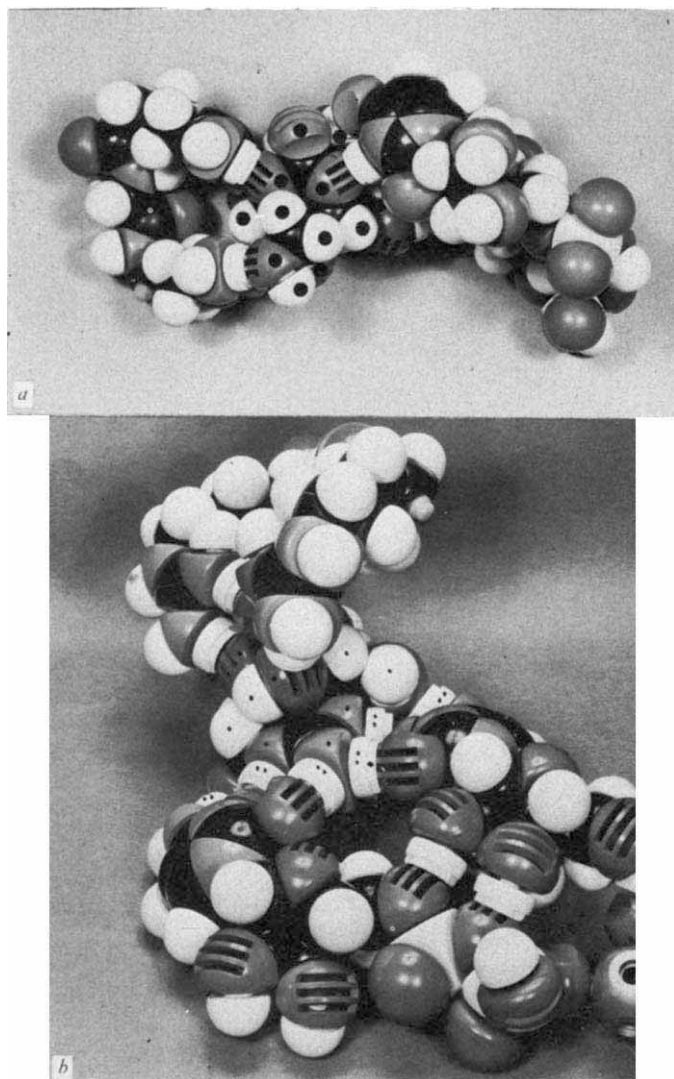


Fig. 1 *a*, Complex of tetrodotoxin (un-ionized) with two separated complementary strands of polypeptide and UMP molecules; *b*, the complex between TTX and two molecules of CDP. The particular amino-acid sequence of the protein shown is Arg-x-Arg-x-Gln. The Gln-tetrodotoxin double hydrogen bond is at the back. The tetrodotoxin-CDP links have two dots.

The molecule of tetrodotoxin is essentially a globule studded with O and OH groups with a protruding tongue—the guanidine group. There are two possible amino-acid-base pairings. (i) The un-ionized form (in a lipophilic microenvironment) could bind to two molecules of UXP on one side and to two glutamine moieties on the other (Fig. 1*a*). This uses nine out of twelve of the available binding groups on the tetrodotoxin molecule and 9/10 of the bonding groups in the site. (ii) The ionized form can bind to two molecules of CXP on one side as follows: cytidine O and N: to guanidine NHs, and NH₂ to the adjacent hydroxyl O on each side of the guanidium tongue (Fig. 1*b*). In this case each cytosine must bind to its neighbour by 2OH to 50, and 30H to phosphate O and there is convergent rotation at the cytosine-ribose bond. The amino-acid side can be various combinations of glutamine and arginine (both complementary to cytosine). This uses ten out of twelve of the bonding groups on tetrodotoxin and all ten of the bonding groups in the site.

Thus, if the nucleoside-protein complex in axonal and muscle membrane was based on these pairs, one can see that when the strands are separated (open Na⁺ channel) the molecule of tetrodotoxin could slip between them and tie them together in this highly efficient manner.

Examination of a molecular model of BTX discloses a possible relationship to the nucleoside side of a base-amino-acid gate (Figs. 2*a* and *b*). The molecule is essentially T-shaped. It bears polar groups on the under surface of the horizontal bar of the T which are complementary to polar groups on the bases. On one side the OH, O, OH grouping is complementary to uracil and on the other side the NH group can bind uracil or cytosine. The stem of the T now slips in between the pyrimidine rings of the two bases with extensive lipophilic and van der Waals' contacts. But in particular, as may clearly be seen in Fig. 2*b*, when the plane of the pyrrole ring is aligned at right angles to the plane of the pyrimidine rings, the 2 and 4 (and any 5) alkyl groups (methyl or ethyl) make extensive lipophilic contacts with the C5 and C6 hydrocarbons of both bases. Finally the (acidic) pyrrole NH group makes a hydrogen bond with a phosphate O. Thus the polar groups on both bases are effectively blocked, the two amino-acids on the other side of the gate cannot be bound and the gate cannot close. The side of batrachotoxin presenting "inwards" is largely lipophilic and thus the Na⁺ channel is kept open. It is possible that a charge transfer reaction is also involved.

This formulation is in accord with the structure-activity relationship data. Removal of the 2 alkyl group of batrachotoxin greatly reduces activity and N-methylation of the pyrrole N virtually abolishes it⁵. On the other hand, addition of a 5 methyl group doubles activity (*vide* locus for this in Fig. 2*b*), whereas 5-substitution by a hydrophilic group (acetyl)

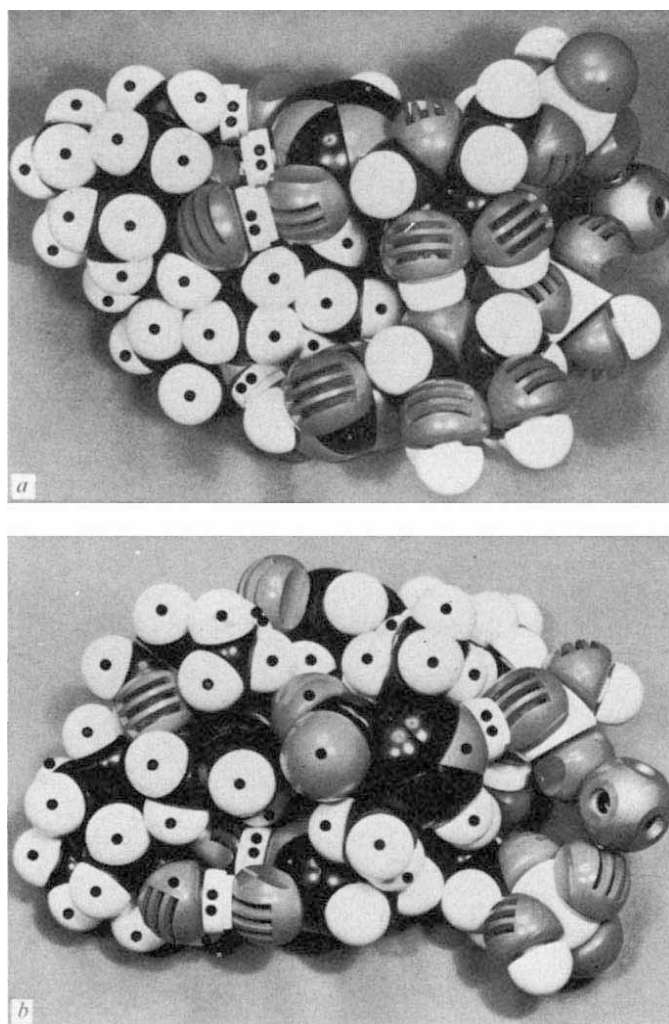


Fig. 2 *a*, The complex of batrachotoxin and two molecules of UMP. The hydrogen bonds described in the text have two dots. *b*, The other side of this complex.

is unfavourable (1/250). Removal of both the 2 and the 4 methyl groups virtually abolishes activity⁵.

Thus both these threads of evidence suggest that the "gate" of the Na⁺ channel in axonal and non-junctional muscle cell membrane is composed of at least two amino-acid-base pairs. The channel could of course include several such pairs connected together in some form of array, and indeed tetrodotoxin and batrachotoxin seem to act at different loci in it. The gate, for example, may consist of the series CCU, or CCUU, or CC . . . UU. The ionized form of tetrodotoxin could bind to CC and close the channel and batrachotoxin could bind to CU or UU and prevent closure of the channel. Thus TTX could prevent the action of batrachotoxin (as it does), but the two molecules would be acting at partially or wholly different sites⁶.

The hypothesis would predict the occurrence of uracil and cytosine nucleotides in these membranes and that the membrane protein concerned has a repeating amino-acid sequence with glutamine and arginine elements. The chemical similarity between the barbiturates, which block axonal conduction, and uridine is noteworthy.

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Effect of Microamp Electrical Currents on Bone *in vivo* and its Measurement using Strontium-85 Uptake

Bassett, Pawluk and Becker¹ reported that they had observed marked bone production in the femora of dogs 3 weeks after insertion into the bone of implants which produced a few μ A of direct current through platinum-iridium electrodes. This work was repeated by O'Connor and colleagues², who were able to report "general confirmation" of Bassett's results, and who found difficulty in assessing the degree of bone growth. We have tried to reproduce the results reported by Bassett¹ in rabbits, and to develop a precise method of assessing the degree of bone growth.

The implants used were similar to those of Bassett¹ and each contained a 1.4 V mercury cell in series with a 39 kohm resistor. These were encapsulated in bone cement from which protruded two platinum-iridium electrodes 1 mm in diameter, about 1 cm long and spaced 9 mm apart. The nominal short circuit current of 36 μ A was reduced to about 3 μ A by polarization effects when the electrodes were inserted into prepared holes in the femur.

Adult white rabbits of the Hyllyne Farm strain weighing approximately 4 kg were used. In each, an active implant was made in one femur, and an inactive control implant (containing no battery) was made in the other. The operations were carried out using 'Fluothane' anaesthesia with an antibiotic cover. Every effort was made to reduce surgical trauma, and

the final technique involved the drilling of straight parallel 1 mm holes 9 mm apart through the cortex of both sides of the bone using a stainless steel template. The electrodes fitted snugly into these drill holes and were cut to end flush with the periosteum on the farther side of the bone.

The rabbits were killed at 3 weeks and both femora were dissected out. Analyses were made in all cases where the implants had remained firmly *in situ* and where there had been no battery leakage. The bones were examined grossly, by radiography, and histologically. In all cases, new bone was found in the area of the implant, both in the medullary cavity of the femur and in the sub-periosteal areas. No difference could be detected by these examinations between the active and the inactive implants or between the anodes and the cathodes of the active implants.

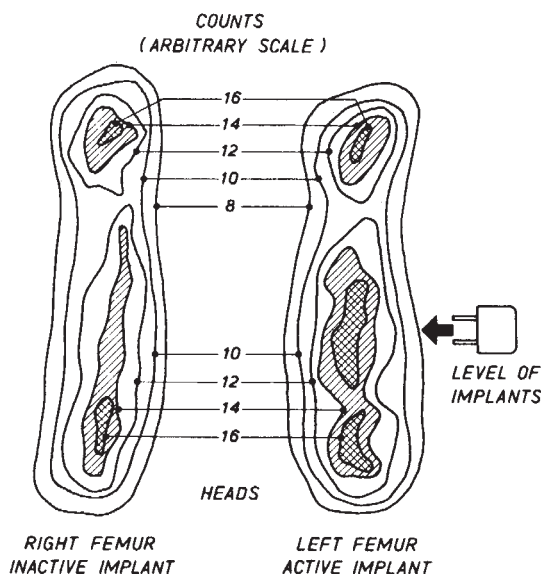


Fig. 1 An isocount presentation of a typical scan. (The actual colour scan is not suitable for reproduction.) Two 5 inch sodium iodide crystal detectors in opposition viewed the specimen through 55 hole collimators (FWHP 1.55 cm). The scanning speed was 2 mm/s, print-out being every 3 mm.

For precise estimation of the degree of this bone growth we used strontium-85 in a scanning procedure. This isotope has been shown to be absorbed into bone with a direct relationship between bone formation and isotope uptake³. At this stage of our work, we have not tried to distinguish between "apposition" and "accretion", but have used the presence of ⁸⁵Sr as a measure of the amount of calcium hydroxy apatite deposited both in the existing matrix and between new collagen fibres⁴.

The standard programme for each experiment is now as follows. Day 0: random implantation of active and inactive implants; day 7: intravenous injection of 75 μ Ci of ⁸⁵Sr; day 21: animal killed, dissected and radioisotope scanning procedure carried out. This schedule should be somewhere near optimum for judgment of bone growth without too much radioactive decay taking place between injection and assay. Femora were scanned in pairs on a commercially available radioisotope scanner which was capable of printing a numerical value representing the summed radioactivity in a chosen interval along each traverse of the scanner. Thus, a relatively quantitative picture was built up as the scan proceeded, and isotope uptake in corresponding sites could be reasonably compared by simple addition of the numbers in each site. Similarly, by adding the numbers in each traverse, an uptake profile could be plotted for the whole length of each bone (Figs. 2 and 3).

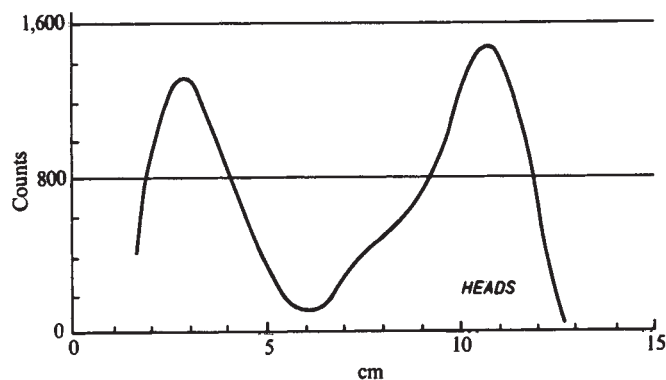


Fig. 2 The natural uptake profile averaged for a pair of femora without implants.

From a consideration of the counts involved, it was concluded that differences in the resultant curves were not due to statistical fluctuations in the measuring technique but to real small differences in bone production.

In the final series of eighteen operations, the following results were obtained: for seven animals, most bone growth was near the active implant; for nine animals, most bone growth was near the inactive implant; one animal showed no detectable difference, and one died from paraplegia. No difference was detectable between the radioisotope uptakes at the site of the anode and the cathode on the active implant side.

This corresponds with the findings of O'Connor *et al.*², whose data showed that there was more growth on the side of the inactive implant nearly as often as on the side of the active implant; the difference between the growth of bone at the anodic and cathodic ends of the active implants was small relative to the total amount of bone growth even when an inactive implant was used.

Our experiments have confirmed Bassett's observation that the magnitude of the available current is limited to a few μA by polarization phenomena in the body tissues. This means that the battery voltage and the magnitude of the series resistor control the current but little. This is implicit in the letter of Lavine and Lustrin⁵, who chose a 3 μA current to show that heating effects are negligible. It is therefore difficult to see the advantage of being able to monitor this current *in vivo*, and Lavine and Lustrin's statement that the experimental model can be varied easily as to the amount of current delivered, by changing the battery and/or resistance, must be qualified in the light of current limitation by polarization.

It has not yet been established whether higher direct currents lead to greater bone formation response, and it is doubtful

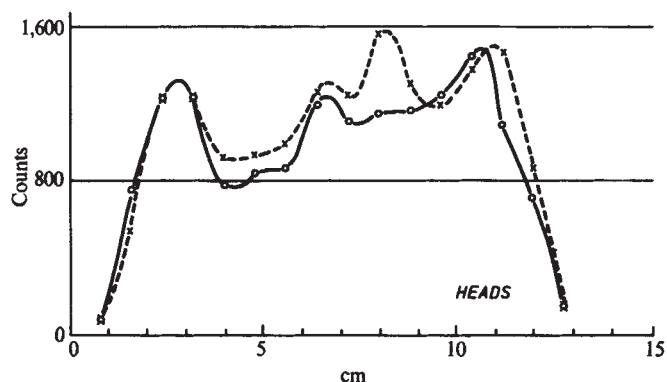


Fig. 3 Natural uptake profile averaged for a pair of femora with implants. $\circ$ — $\circ$, Right femur (inactive implant); $\times$ — $\times$, left femur (active implant).

whether experiments can be designed to show this if polarization phenomena are unavoidable. But if fluctuating current is used, at such frequencies that polarization has a negligible effect, it may be possible to produce currents several orders of magnitude greater than the direct currents previously used. Modern techniques using microcircuits encapsulated in bone cement along with the batteries are making this approach feasible and work along these lines is now being carried on.

We therefore conclude that no consistent evidence of increase in bone growth at one electrode over that at the other electrode of an active implant can be shown by gross examination, histology or radiology; this conclusion is borne out by the new radioisotope method of assessment devised for this series of experiments. Any bone growth resulting from the small direct currents used is very small compared with the growth resulting from trauma, although there may be a case for designing experiments to (a) achieve larger currents using a.c. to avoid polarization effects and (b) obtain a much better placement of electrodes to avoid excessive trauma.

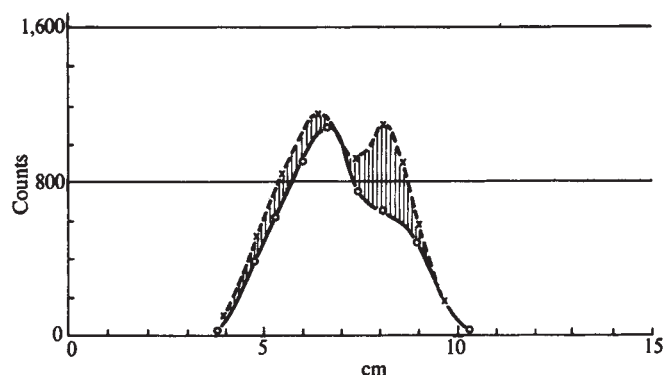


Fig. 4 Graphical representation of the estimated bone growth in excess of the natural uptake profile. It was obtained by normalizing the scales of the natural uptake profile curve of Fig. 2 to those of Fig. 3 so that the large peaks corresponding to the head and condyles of the bones coincided, and subtracting the ordinates at corresponding points along the bone length. The two resulting curves were superimposed to allow for slight differences in implantation site and to allow for a visual comparison. The areas under each curve represent excess bone growth due to trauma plus any electrical effects. $\circ$ — $\circ$, Right femur (inactive implant); $\times$ — $\times$, left femur (active implant).

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Ramapithecus wickeri Mandible from Fort Ternan, Kenya

THE portion of a hominoid mandible (KNM*-FT 45) found in 1962 at Fort Ternan in Kenya has been referred provisionally by Leakey¹ to *Dryopithecus (sensu strictu)*. Simons² has agreed essentially with this. Other primates found at Fort Ternan include the maxilla fragments of *Ramapithecus wickeri* (Leakey) 1962 (ref. 3), several individuals of *Pliopithecus*, and several isolated teeth attributed to *Proconsul* and *Oreopithecus*. By size alone the mandibular specimen (KNM-FT 45) can be distinguished from all these except *R. wickeri*. A detailed examination of occlusal relationships between the upper teeth in the maxilla of *R. wickeri* (KNM-FT 46)[†] and the two premolars in KNM-FT 45 suggests that the two specimens belong to the same individual; and even if this is not so they definitely belong to the same species. Simons⁴ and Pilbeam⁵ have argued that Leakey's taxon *Kenyanthropus wickeri* is a junior synonym of *Ramapithecus punjabicus* (Pilgrim) 1910 (ref. 6), but the evidence indicates at least a species difference, and this is the position I take here.

Specimen KNM-FT 45 was found on the surface of the gully leading down from the exposures at Fort Ternan and so its stratigraphical relationship with the other fragments of *R. wickeri* is unknown. The mandible represents the first known specimen of an Upper Miocene hominid that has the crowns of the lower premolars intact and has details of the symphysis and root sockets of the canine and lower incisors.

Specimen KNM-FT 45 is a portion of the left body of the mandible with part of the mental region preserved nearly to the midline of the symphysis. It has the crowns of both premolars in good condition, except for a chip off the distolingual border of the fourth premolar, and also has the mesial root of the first molar and the root socket of the canine. The lateral surface of the body and mental region of the mandible is intact with the exception of the eroded region of the incisor alveolar process, in which only the tip of the root of the lateral incisor and part of the socket of the central incisor can be seen. Medially part of the cortex has been eroded, but the general conformation of the bone can still be seen. The posterior part of the inferior border of the mandible has been a little displaced laterally and superiorly, buckling the lateral cortex.

The symphysis of the mandible is shallow and long with a strongly developed inferior transverse torus. The torus curves sharply medially from its point of departure at the base of the mandible beneath the first molar. The inner border of the symphysis slopes posteriorly at a shallow angle to just above the genial pit, but the region of the pit is missing and its structure cannot be determined. The outer border of the symphysis is also shallow and lacks any kind of angle or eminence. The conformation of the symphysis is shown in Fig. 1. A cross-section of a pongid symphysis is also shown to emphasize the essential difference of all other hominoids from the condition seen in *R. wickeri*. Hominoids always have the vertical height greater than the horizontal length in contrast to *R. wickeri*, which combines the shallow symphysis with a great posterior extension of the torus. The body, like the symphysis,

is relatively low and robust. The estimated measurements for height and thickness at the first molar are 21.2 mm and 11.7 mm respectively, giving an index of 55.2. The conformation of the body of the mandible at the distal end of the first molar has nothing to suggest that an inferior torus is present, so abruptly does the torus arise.

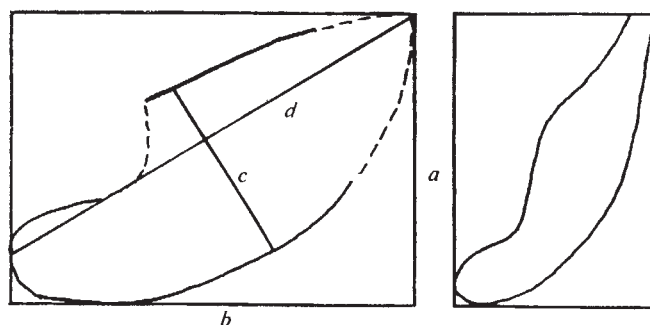


Fig. 1 Symphyseal cross-sections of KNM-FT 45 on the left and an orang-utan (KNM MP 7 Po (1)) on the right. Drawn to the same vertical size. The dimensions are (a) 21 mm; (b) 30 mm; (c) 14 mm; and (d) 35 mm. The dotted line signifies reconstruction.

The parts of the root sockets of the canine and incisors that are present show that these teeth must have been very small, comparable in size with the larger *Limnopithecus* species from Kenya. The alignment of the roots is arcuate, the incisor roots being only slightly anterior to the third premolar. The position of the incisor root sockets suggests that either these teeth were procumbent or they had strongly anteroposteriorly curved roots, or both. The canine dimensions estimated from the root socket are given in Table 1.

Table 1 Measurements of Dentition

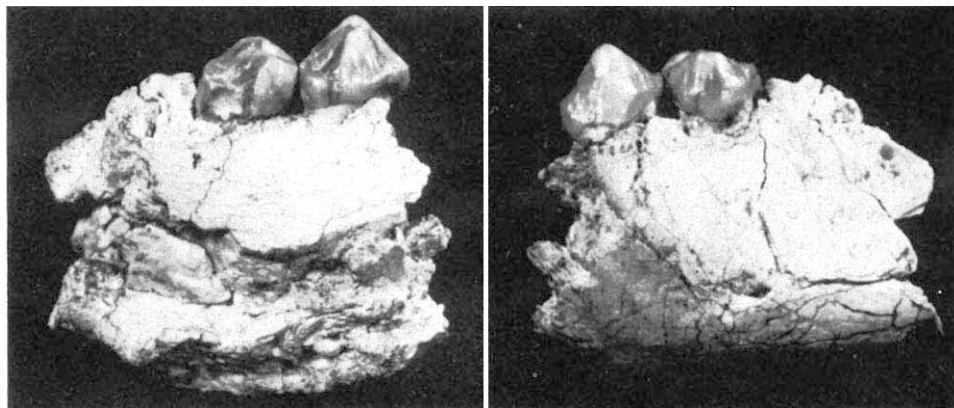
	C (root socket)	P3
Maximum length (L)	7.6	10.9
Perpendicular breadth (B)	5.2	6.6
Index B/L × 100	68.4	60.6
Length of root	15.0	
		P4
Mesiodistal length		8.4
Buccolingual breadth		8.6

The third premolar is semi-sectorial (Fig. 2), a feature Leakey predicted for *R. wickeri* in 1962 (ref. 3). The wear facet along the mesial border of the principal cusp fits the corresponding facet on the distal border of the upper canine of the left maxilla of *R. wickeri*. There is incipient development of a second cusp on the ridge running distolingually from the principal cusp. The tooth is set at the relatively large angle of 60 degrees to the axis of the fourth premolar and root of the first molar, and the second cusp is thus brought into occlusion mesially. This may be the start of a molarization process of the third premolar, a process also indicated by the presence of a large talonid and a moderately large lingual cingulum. The fourth premolar is bicuspid. It has a relatively large mesial fovea, and distally the talonid is large and shallow, so that the tooth appears relatively long. The wear faceting on the talonid corresponds to that of the protocone of the upper fourth premolar of the *R. wickeri* maxilla, the two teeth fitting very tightly together. There is a very large distal contact facet made by contact with the first molar, the roots of which are pressed right up against the end of the premolar. The dimensions of the premolars are given in Table 1. The length/breadth index for the third premolar is similar to the values for the larger species of *Proconsul*.

* Kenya National Museum.

† Field number F.T. 1272

Fig. 2 Occlusal-lingual view of KNM-FT 45 on the left, buccal view on the right. Natural size.



The occlusal relationships between the teeth of specimens KNM-FT 45 and 46 are shown in Fig. 3. The upper canine has been reset from its position high up in the alveolus to near the one described by Leakey in 1962 (ref. 3), a move also suggested by Simons. Subsequently, when the teeth are locked in occlusion they fit together closely and there is little free movement. It is thus very likely that the two specimens belong to the same individual.

There are several points of similarity between the KNM-FT 45 and the Indian *Ramapithecus punjabicus* mandibles although in none of them can the structure of the symphysis be seen. The mean breadth/depth ratio of the mandible at the first molar for three specimens of *R. punjabicus* is 55.8, very close to the value for *R. wickeri*. Simons shows⁴ that the medial border of the mandible of YPM 13814 turns inward slightly towards the symphysis beneath the first molar, the condition seen in the Fort Ternan mandible. The Domeli mandible, referred by Pilbeam to *Ramapithecus*⁷, has an inferior transverse torus originating below the fourth pre-molar, and this and its deep, narrow body and symphysis are typically pongid so that they cannot have functional affinities with this taxon.

The symphysis of KNM-FT 45, which extends back to the level of the first molar, is neither hominid nor pongid. The position and size of the canine and incisors indicate a strongly compressed anterior region of the mandible, and this corresponds to the evidence for shortening of the face present in all the maxillary specimens of *Ramapithecus*⁸. But it is unlike Miocene pongids in which the relatively large canine and incisors project well anterior to the cheek teeth. The possibility that the incisors were procumbent is puzzling, but this must await the finding of more complete specimens before it can be discussed in functional terms.

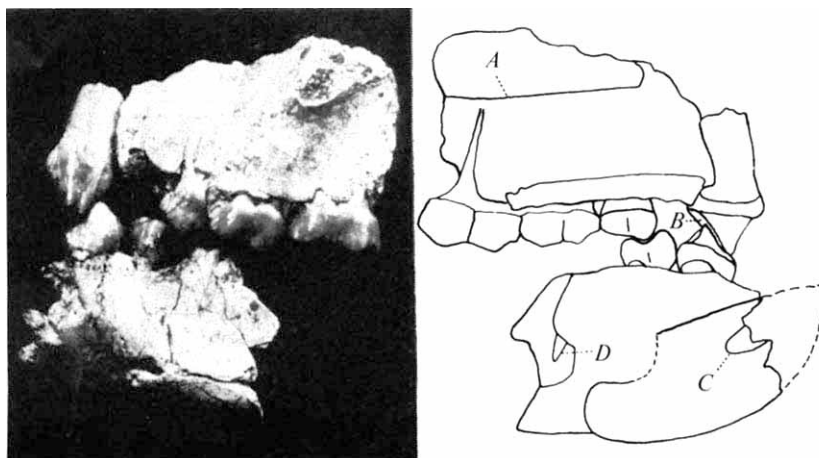
No one element in morphology of *Ramapithecus* can be absolutely characterized as hominid as distinct from pongid, but it is the assemblage as a whole that is significant^{8,9}. The

semi-sectorial premolar, the long inferior transverse torus, and the possibly procumbent incisors in *R. wickeri* are generally considered pongid characters, but these are not necessarily incompatible with the overall trend toward later Hominidae. The anomalies cease to be such when the combined mandible and maxilla are considered in functional terms, for together they form a consistent and integrated unit that is apparently adapted for increased lateral grinding and more powerful chewing. (This would not be unexpected in a hominid ancestor, and is a good illustration of mosaic evolution.) The evidence for this is briefly outlined here as follows.

(1) The forces acting on the body of the mandible are those resulting from the direct pull of the muscles, and those from the dissipation of compressive forces generated in occlusion. The first varies according to the muscles most active at the time. The ipsilateral action of the masseter or medial pterygoid muscles¹⁰ act both to raise the mandible and to grind the mandibular teeth sideways against the frictional resistance of the teeth of the maxilla; the opposite action of the muscular and reaction forces produce a twisting couple, the moment of which is the greater vertical distance between the insertions of the muscles and the occlusal plane, or in other words the deeper the mandible. There is therefore a mechanical advantage in having a reduced depth of the body of the mandible where there is a strong lateral component in chewing. A low and robust mandible is also an advantage in the dissipation of forces which are set up in the mandible as a result of sideways chewing, with the buttressing of the body on either side of the roots¹¹. This is seen in both species of *Ramapithecus*⁴.

(2) The three forces acting on the symphysis are the reaction forces generated through occlusion of the canines and incisors at the symphysis, the muscular forces of the body of the mandible as they are transmitted through the symphysis, and the anteroposterior shearing forces generated during axis rotation of the mandible. The first of these cannot be dealt with here

Fig. 3 KNM-FT 45 and 46 in occlusion. On the left is a photograph showing the buccal view, and on the right is a drawing made from a photograph of the lingual view. A, Approximate line of floor of maxillary sinus; B, distal wear facet on the upper canine; C, root socket of the central lower incisor; D, tip of distal root of the first lower molar. The dotted line signifies reconstruction.



as the crowns of the incisors are not known for *Ramapithecus*. The muscular forces which have different effects on the body of the mandible all have similar effects when transmitted across the symphysis. For example, during one sided chewing when the masseters are active on both sides, the force of the contralateral masseter passes through the symphysis to produce a twisting couple. The moment of this couple is determined by the depth of the body of the mandible where the forces are being generated and the strength of resisting this at the symphysis is greater if (a) the symphysis is relatively deeper than the body; (b) the surface area of the symphysis is enlarged; and (c) the buttress of the symphysis is mechanically efficient. All these conditions are met in *R. wickeri* showing it to have had a symphysis well adapted to resist lateral strains. The shearing force generated by the lateral pterygoids in shifting the axis of rotation from one condyle to the other^{10,12} is resisted by anteroposterior buttressing or lengthening of the symphysis¹³, which might explain the great lengthening of the symphysis of the Fort Ternan mandible.

(3) The zygomatic process on the *R. wickeri* maxilla is strongly laterally flared, indicating the same condition was present in the missing zygomatic arch. This suggests a large space available to accommodate the temporal muscle and this in turn indicates powerful centric occlusion¹⁴.

(4) The position of the root of the zygomatic process above the first molar on the maxilla of *R. wickeri*¹ and the steepness of the ascending ramus on the *R. punjabicus* mandibles⁸ both suggest that the line of action of the anterior fibres of the temporal muscles was nearly vertical. The superficial fibres of the masseters must have been directed forwards to their attachment on the zygoma so that they functioned as protractors of the mandible¹⁵. The masseter would also be acting at a greater distance from the mandibular condyle and would therefore have a greater power arm about the jaw joint, a contribution to more powerful chewing¹⁴ that parallels the evidence for enlarged and vertically acting temporal muscles.

(5) The alveolar process of the less complete right maxilla³ of *R. wickeri* is greatly inflated. The distance from the alveolar margin to the floor of the maxillary sinus is well beyond the range in size seen in *Proconsul* and the great apes¹⁶. The roots of the upper molar in *R. wickeri* are fully supported all round, whereas the usual condition in hominoids is for the floor of the maxillary sinus to be excavated between the roots of the molars. It seems likely that this is an adaptation for dissipating lateral chewing strains in the same way as that described earlier for the mandible.

(6) Some features of the dentition indicate a lateral element in chewing, including the transverse wear faceting on the molars and premolars of the mandible and maxilla. The low relief of the cusps on the molars and premolars and the reduction in size of the canine both indicate a reduction in the locking mechanism of the dentition and are essentially correlated with increase in lateral grinding. It is not yet clear, however, whether these changes came about in hominids in response to some other pressure so that the dentition was preadapted to lateral grinding or whether they occurred as a direct response to change in diet involving an increased lateral component in chewing^{17,18}.

These factors suggest that powerful lateral grinding was developed in both species of *Ramapithecus*. This and the accompanying reduction of the incisors and canines relative to the molars parallel the functional complex of the Madagascar fossil lemur *Hadropithecus* which Jolly has recently interpreted as a small object feeder^{11,17}. He has also pointed out, however, that if this functional complex is seen to have evolved three times independently in primates it could also have evolved twice independently just within the hominoids, in *Ramapithecus* and in the later australopithecines. Although this is possible, the most straightforward hypothesis at present is to link these two functionally similar taxa into one phylogenetic lineage, the Hominidae.

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Chromosomal and Serological Studies of the Caenolestidae and their Implications for Marsupial Evolution

SIMPSON¹ recognizes five superfamilies of living marsupials, three of them Australasian (Dasyuroidea, Perameloidea, Phalangerioidea) and two American (Didelphoidea, Caenolestidae), and regards none of these as specially related at a higher categorical level. Since Thomas's² description of *Caenolestes obscurus*, however, the affinities of the American marsupial superfamily Caenolestidae have been in doubt largely because of the diprotodont incisors, a feature of their dentition that caenolestoids share with the Australasian phalangeroids. Thus, some authors²⁻⁴ have proposed a special relationship between these groups, while others^{5,6} have rejected the dental similarities as convergent and grouped caenolestoids with the polyprotodont marsupials which include both American and Australasian forms; still others^{7,8} treat Caenolestidae as representative of another principal group of marsupials. The question of caenolestoid affinities is thus a problematical one, to which morphological study has contributed little since Osgood's study³. Chromosomal and serological analyses might be expected to be relevant as there have been extensive studies of the chromosomes⁹⁻¹² and serum proteins¹³ of four of the marsupial superfamilies. We present here, for the first time, data on the karyotypes and comparative serology of the Caenolestidae, encompassing three of the seven extant species representing two of the three genera. Details of the localities, ecology and behaviour of these species will be published elsewhere.

The chromosomes of *Caenolestes obscurus* (male, field number JAWK 342, Paramo de Purace, 3300m Depto. Cauca, Colombia) and *Lestoros inca* (male, field number JAWK 460, 25 km by road North-West Ollantaytambo, 3530m Depto. Cuzco, Peru) are shown in Fig. 1. The chromosomes of *C. fuliginosus*, not shown, are very similar to those of *C. obscurus*. Each species has $2n=14$ with a simple XX/XY sex chromosome system; the Y chromosome is typically minute.



Fig. 1 The somatic chromosome complement of *Caenolestes obscurus* (male, upper row) and *Lestoros inca* (male, lower row). The chromosomes are from cell divisions in marrow tissue treated with colchicine *in vivo*. A small achromatic region can usually be found near the end of the short arm of the smallest submetacentric chromosome in both species, although it does not show in this figure.

In the Australasian superfamily Dasyuroidea, all species have seven pairs of chromosomes, the autosomes including three pairs of large metacentric or submetacentric chromosomes, one pair of medium sized near-metacentric chromosomes and two small pairs of chromosomes of which one has a satellited short arm¹². Chromosome numbers and morphologies vary considerably within the other three superfamilies, namely the American Didelphoidea and the Australasian Phalangeroidea and Perameloidea; but they all include species which have autosomes similar to those of the Dasyuroidea. Fig. 1 shows that this complement is also characteristic of the caenolestids and we suggest that this confirms the hypothesis¹² that $2n=14$ with this form of complement is ancestral for the marsupials. It is uncertain when the marsupial superfamilies diverged, recent estimates varying from the Lowest Tertiary¹⁴ to the Jurassic¹⁵. Whichever is correct, it is clear that in some species of three superfamilies, and in all known species of the two other superfamilies, the karyotype has remained remarkably stable.

Although chromosome studies show that the caenolestids share a primitive karyotype with members of the other marsupial superfamilies, they do not indicate the phyletic relationships among those superfamilies. Earlier serological studies have shown that all Australasian marsupials grouped more closely to each other than to the few didelphid marsupials available for study at that time¹³. This result has been confirmed by tests on a wider range of didelphid material collected in South America, and caenolestids have also been examined serologically. Antisera were prepared in rabbits against sera of several didelphid species and *Caenolestes obscurus*; these, and several antisera previously made against Australasian marsupial sera, were reacted with a range of sera from Australasian and American marsupials in immunoelectrophoretic tests on microscope slides. Reactions were performed with both unabsorbed antisera and aliquots of antisera absorbed in various ways with heterologous sera. These tests indicated that caenolestids have no greater similarity to any Australasian marsupial superfamily examined than to the didelphids; in fact, the data suggest that caenolestids are less like any of the other superfamilies of marsupials than those superfamilies are like each other. Thus serology does not seem to support a separation of living marsupials into Australasian and American stocks, but suggests that the caenolestids diverged from the principal line of marsupial evolution before the separation of didelphids and Australasian marsupials. Such a conclusion, however, assumes that serological affinity strictly reflects propinquity of descent, and furthermore, because only two closely similar, modern genera of caenolestids were studied, it leaves little allowance for variation within groups or for aberrant results. But the interpretation does not conflict with the palaeontological data, which indicate that the caenolestids were already highly specialized in the earliest Tertiary¹⁴.

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Suppression of Fighting Behaviour in Rabbits by Paired Emergence from Anaesthesia

TINBERGEN¹ has pointed out that aggression is the most directly lethal of human behaviours and that "we must pursue the biological study of animal behaviour for clarifying problems of human behaviour of such magnitude as that of our aggression". Aggressive behaviour in animals can be suppressed by many drugs² but rarely for more than a few hours³. Possible exceptions are chlorpromazine and some of its derivatives^{4,5} and chlordiazepoxide⁶. We wish to describe a prolonged suppressive effect induced by allowing aggressive rabbits to recover in pairs from barbiturate anaesthesia.

A prolonged abolition of aggressive behaviour in male rabbits after surgical anaesthesia with sodium amobarbital was first observed by Dr S. W. Warren and Ruth A. Boak in 1942 but has not been reported. During syphilis experiments, male rabbits were housed singly because pairing invariably led to severe attacks with castration and death of one rabbit. On one occasion, however, two rabbits were left in a cage together to recover from amobarbital anaesthesia. Surprisingly, the usual attack behaviour did not occur after recovery of consciousness and the pair remained docile for as long as they were caged together. This unexpected reversal of behaviour permitted routine caging of male rabbits in pairs.

We now report a confirmation of this observation and call attention to possibly related observations with chickens⁷, cats and dogs⁸. The male rabbits in this study were pre-selected for aggressive behaviour on grouping in pairs. Although both traumatic behaviour (clawing and biting) and non-traumatic behaviour (foot-thumping and copulatory movements) were observed, only traumatic behaviour was used as a criterion for selection.

In a preliminary experiment with an aggressive pair of Dutch rabbits, anaesthesia with halothane or methoxyflurane did not

suppress aggression but sodium pentobarbital anaesthesia (20 mg/kg, intravenously) suppressed attack behaviour for the ensuing 14 weeks. Re-isolation for 2 weeks, followed by re-grouping, resulted in attacks within 2 min. Our experiment was carried out in covered outdoor quarters in southern California between September and January with the rabbits (2.7 to 4.5 kg) visually isolated from each other in metal cages (89 × 76 × 76 cm) with wire mesh floors. The adult male New Zealand rabbits (California Caviary, Inglewood, California) were ordered with the specification that they be "aggressive"; approximately half of the rabbits received could be grouped in pairs to meet our criterion. The criterion was two bouts of vigorous clawing or strong biting that removed fur, with elicitation of retreat or reprisal by the attacked rabbit, when an intruder was placed in the cage of a resident for 5 min. The thirteen pairs that met the criterion were then exposed to five daily bouts to stabilize the latency of attack, with recordings of latency to first attack and frequency of attacks per 5 min bout. After the fifth stabilization bout, the thirteen pairs were divided into three groups originally matched for mean attack frequency. During the experiment four pairs had to be eliminated because of illness or trauma, resulting in differing pre-anaesthesia values (Fig. 1). All test rabbits were anaesthetized with sodium pentobarbital (35 to 45 mg/kg, intraperitoneally); the members of each pair were anaesthetized simultaneously. The induction time to loss of the righting reflex was 17 ± 5 min and the time to recovery of the righting reflex was 95 ± 45 min. Group A rabbits (four pairs) were paired in the resident's home cage while anaesthetized, during recovery, and for 7 days thereafter. Group B rabbits (two pairs) were isolated in home cages during and after recovery. Group C rabbits (three pairs) recovered together and remained together 24 h, after which each pair re-isolated.

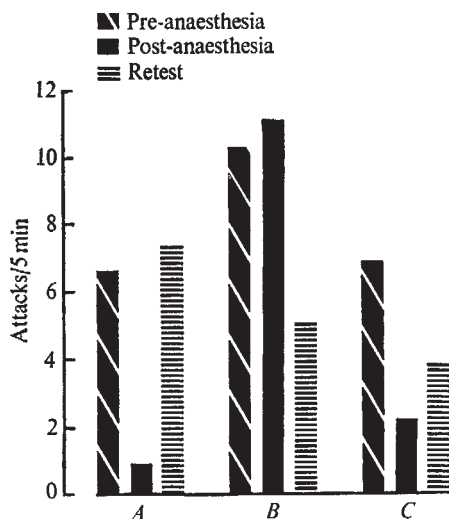


Fig. 1 Mean number of attacks per 5 min pairing. Group A (four pairs) recovered from anaesthesia together and remained together; Group B (two pairs) recovered separately and remained apart; Group C (three pairs) recovered together and were re-isolated 24 h later. Significant differences among the groups were indicated only during the post-anaesthesia stage ($P < 0.001$, post-anaesthesia; $P > 0.3$, pre-anaesthesia and retest; Kruskal-Wallis test). In the post-anaesthesia stage, group B attacked significantly more often than groups A or C ($P < 0.0005$; Mann-Whitney U test); there is also a small but significant difference between groups A and C ($P < 0.01$, Mann-Whitney U test) suggesting a slightly reduced anti-aggression effect when pairing was for only 24 h after anaesthesia.

Animals were tested for attack behaviour for 7 days after re-isolation of group C. Group A pairs were examined daily for traumatic damage inflicted during the preceding 24 h and were also observed daily for 5 min. Group B and C rabbits were paired daily for a 5 min test bout. After 7 days of paired caging, group A intruders were re-isolated in their home cages.

Four weeks after re-isolation, all groups were given a final series of five daily test bouts.

Recovery from anaesthesia in pairs (groups A and C) reduced post-anaesthesia attacks whereas anaesthesia with isolated recovery (group B) did not. Re-isolation of groups A and C rabbits for 4 weeks resulted in re-emergence of attack behaviour (Fig. 1).

We conclude that when aggressive rabbits recover simultaneously from pentobarbital anaesthesia in one cage, the usual traumatic attack behaviour is suppressed as long as the pair remains together. Furthermore, pairing during recovery and the ensuing 24 h significantly decreases attack behaviour for at least 1 week. Galef⁹ has pointed out the critical role of stimulus novelty in eliciting aggressive behaviour in rats; perhaps the "novelty" of one rabbit to another is dulled during slow emergence from anaesthesia and is abated by the gradual familiarization occurring during emergence.

We attempted to suppress isolation-induced aggression in mice by grouping them in triads while anaesthetized by halothane, methoxyflurane, or sodium pentobarbital; only transitory attenuation occurred in our conditions. Dr R. P. Huemer (personal communication) observed that female mice could be safely caged together in groups of about six during and after recovery from surgical anaesthesia with diethyl ether even though the mice had extensive wounds and bloody skin whereas unanaesthetized wounded mice often attacked each other. We were unsuccessful in suppressing the aggressive display to a mirror of the male Siamese fighting fish (*Betta splendens*) anaesthetized with halothane or tricaine methane-sulphonate.

Suppression of aggressive behaviour for 3–5 days after ethanol has been reported in the domestic chicken⁷. Dr S. W. Warren (personal communication) found that vicious dogs became tractable after surgical anaesthesia followed by gentle handling for 24 h, and suggests that the markedly altered behaviour may represent a form of "imprinting" of the dog upon the friendly experimenter. Dr D. G. DeValois (personal communication) has observed consistent docile behaviour in pound dogs, grouped three or four per cage after anaesthesia for devocalization surgery. Fox⁸ cites reports that fierce cats and dogs became docile to man after intravenous barbiturate anaesthesia and suggests that the effect is "worthy of rigorous clinical evaluation by the veterinarian in practice". Presumably, the animal should be gentled during recovery. A similar clinical evaluation of assaultive human patients may merit consideration.

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BOOK REVIEWS

Polydamna's Drug

Origins of Psychopharmacology from CPZ to LSD. By Anne E. Caldwell. (A Monograph in the Bannerstone Division of American Lectures in Objective Psychiatry.) Pp. xiv+225. (Thomas: Springfield, Illinois, 1970.)

"RARELY if ever has any drug at any time equalled the impact of chlorpromazine on psychiatry, medicine and society." Caldwell's claim is not difficult to justify, for chlorpromazine (CPZ) has revolutionized the treatment of psychoses, enabling many patients to live at home instead of in hospital; the consequent benefits to society have been very great and should not only be counted in economic terms. It is, for example, easier to accept that mental illness has a somatic basis if it can be treated by a drug.

What is the secret of chlorpromazine's success? Dr Caldwell points out that the action of chlorpromazine is the same as that of the drug which Polydamna gave to Helen of Troy. In her own translation of a passage from *The Odyssey*, this is:

"... a drug against sorrow and anger, a drug to suppress despair. Whoever drinks of this mixture will not shed a tear all day long: not even if his mother and father lie there dead; not even if his brother or beloved son is slain before his own eyes while he looks on." What is remarkable about Polydamna's drug is its ability to keep a person calm despite the most extreme provocation. It is this state of mind which was called "ataraxy" by Fabing and Cameron in 1955. Ataraxy, derived from the Greek, means not disturbed, steady, or calm. Ataraxic drugs are also commonly called antipsychotics, anxiolytics, psycholeptics, neuroleptics, or tranquilizers. To avoid this confusion there is much to be said for adopting the term ataraxic. Chlorpromazine is a major ataraxic, effective in treating psychoses, and can be distinguished from the minor ataraxics which are useful in treating neurotic patients.

Psychopharmacology, "the use of drugs to restore mental health and to explore the mind", is not new, but so many false starts were made that Caldwell's claim that the subject really started in 1952 with the synthesis of chlorpromazine is not unreasonable. Some of the false starts are mentioned, but the author's principal task has been to describe for the first time in the secondary literature the fascinating story of the discovery of chlorpromazine. As told by Caldwell, the story has a hero: the French surgeon

Laborit. How is it that a surgeon played such a vital role in the development of a drug for use in psychiatry?

One of the problems a surgeon faces is that of shock, and Laborit began to study this in 1945 with the idea of finding a drug which would inhibit the entire autonomic nervous system, for he believed that shock resulted from an exaggerated organic defence reaction to stress. When Laborit tried the antihistamine drug promethazine in 1949, he found that it was surprisingly effective in the treatment of surgical shock; this phenothiazine derivative produced a kind of "euphoric quietude" which was different from that produced by morphine. It was undoubtedly a central effect and so why not look for other phenothiazines with central activity? Until that time, the pharmaceutical companies had put a lot of effort into making antihistamines with minimum "sedative side-effects", but in 1950 the firm Spécia reversed this policy and took up Laborit's challenge. Using Macht's rope climbing test, it was possible to distinguish the unique central effect of promethazine (its ataraxic effect) from ordinary cortical sedation. By 1951, Spécia had made chlorpromazine, a phenothiazine derivative that combined great central activity with low toxicity and which had little antihistaminic activity.

The first report of the clinical use of chlorpromazine was made by Laborit and his collaborators in 1952. It included a clear description of the ataraxic effect:

"... il ne provoque aucune perte de conscience, aucune altération du psychisme mais seulement une certaine tendance au sommeil et surtout un 'désintéressement' du malade pour ce qui se passe autour de lui." The authors point out that there was a curious resemblance between the deconditioning effect of chlorpromazine and that obtained by the methods of Pavlov. It did not take long for Laborit to persuade his psychiatric colleagues in Paris to try the new drug, and the first reports of its dramatic action in psychotic patients came later in 1952. Delay, for example, described patients who had received the drug thus: "steeped in sweet indifference, they seem separated from their surroundings by an invisible veil". Here, at last, was a drug fit to be compared with Polydamna's!

This is, in summary, the story told by Caldwell. The subject is an important one, but I am not happy about the way it has been told. It appears that the book was derived from several lectures, and it suffers from repetition and

hyperbole. Although well documented (over 300 references) the book contains much that is superfluous and there is a strange mixture of fact and fancy. It is not, therefore, an objective history of psychopharmacology but it can nevertheless be recommended to anyone interested in how one of the world's most important drugs came to be made.

A. D. SMITH

Spiral Arms in Space

The Spiral Structure of Our Galaxy. Edited by W. Becker and G. Contopoulos. (International Astronomical Union Symposium No. 36, held in Basel, Switzerland, August 29–September 4, 1969.) Pp. xiii+478. (Reidel: Dordrecht, 1970.) Hfl. 80.

ONE of the intriguing features of many types of galaxy is their spiral structure. This spiral pattern of trailing arms is found in about half of all galaxies and is evidently a permanent or long enduring property. It could be a permanent imprint on the galaxy or it may be an ephemeral but repeating phenomenon. This is a problem which has intrigued astronomers since the first telescopic observations by Herschel. The study of galactic types was developed by Hubble in 1935 and summarized in his *The Realm of the Nebulae*. It was therefore entirely appropriate that the 50th anniversary of the IAU in 1969 should be celebrated by a symposium on one of the continuing problems in astronomy, entitled *The Spiral Structure of Our Galaxy*.

What exactly is the spiral structure of a galaxy? Each observer will give a different account based on his own assessment of the wide range of patterns found in galaxies. The underlying common feature of spiral galaxies is the existence of elongated spiral arms traced out by gaseous material (neutral hydrogen lanes, ionized HII regions and dust) and stars which have condensed out of this gas within the past 10^7 years or so (OB stars, long period Cepheids and possibly Wolf-Rayet stars and early Be stars). Stars of greater age than this, which incidentally comprise the major part of the mass of a galaxy, are more or less uniformly spread and show none of the spiral structure which characterized their place of origin 10^8 to 10^{10} years ago. The formalized picture of a spiral galaxy beloved of theoretical astronomers is of a double logarithmic spiral starting near the centre of the galaxy and trailing outwards. Indeed this is a perfectly acceptable first order generalization of a complex situation. An actual spiral arm,

however, is far from continuous, showing breaks and concentrations as well as many branches or feathers which are well illustrated in the many photographs reproduced in the proceedings of this symposium. A major category of spiral galaxies has a ring structure in the central regions from which the spiral arms emerge; another group has a central bar which is the starting point for a double spiral arm pattern.

The Basel symposium brought together most of the optical, radio and theoretical astronomers who were working in the field of galactic and extragalactic structure. On the observational side the neutral hydrogen data give the best description of the spiral structure of our galaxy; two radically different interpretations of the observations have been made which have yet to be reconciled. As a consequence it is not at all clear how to join the various elongated features together in order to outline the possible continuous spiral structure. Optically, more data have become available in recent years and this has led to a good picture of the spiral structure near the Sun.

The renewed interest in the theoretical interpretations of spiral structure in galaxies was evident in the large number of papers presented on this topic at the symposium. The gravitational interpretation of the spiral structure seems now to be firmly established and has taken over completely from the magnetic field interpretation which had been popular in various forms for some 20 years. The general magnetic field is now known to have a strength of only 2–3 microgauss and is therefore unable to exert sufficient mechanical force to have any significant influence in producing spiral structure. It does, however, have an important role in influencing local galactic structure and, of course, in producing dust grain alignment, synchrotron radio emission and a number of other observable effects.

This book emphasizes the large amount of high quality observational material available, and demonstrates that a considerable amount of success has been achieved in explaining many of the observed phenomena.

The editors are to be commended for their speedy and pleasing production of this volume which will be valuable to workers in this and allied fields of astronomy.

R. D. DAVIES

Life of Gauss

Carl Friedrich Gauss: a Biography. By Tord Hall. Translated from the Swedish by Albert Froderberg. Pp. 176. (MIT: Cambridge, Massachusetts and London, December 1970.) £3.75.

THIS book is rather disappointing, for it adds little to our knowledge of Gauss. Tord Hall seems more concerned to

instruct the reader in mathematics than to write a historical study of Gauss's work. His procedure is to indicate Gauss's ideas, say, on the geometry of curved surfaces, and then to provide illustrations of the mathematical techniques by means of examples. The primary purpose of this book is not a historical analysis of Gauss's mathematical achievements, and its success should therefore be judged with respect to the terms which the author has tried to satisfy. To me, the mathematical sections of this book are cogent, and are comprehensible to readers of modest mathematical attainment. The book contains an outline of Gauss's diverse fields of interest leavened by a pleasant biographical portrait, and serves as a useful introduction to a very difficult subject. Nevertheless, the sketch of Gauss's scientific personality in this book is far too anecdotal.

P. M. HEIMANN

Homage to Richard Rado

Studies in Pure Mathematics: Papers in Combinatorial Theory, Analysis, Geometry, Algebra, and the Theory of Numbers. Edited by L. M. Mirsky. Pp. viii+276. (Academic: London and New York, January 1971.) £5.00.

THE 26 papers in this volume are offered to Richard Rado in celebration of his sixty-fifth birthday. Such a birthday present is a tribute to the distinction of the mathematician and also to the abundant friendliness of the man. Rado was born in Berlin and studied at the universities of Berlin, Göttingen and Cambridge. After appointments in the mathematics departments of the University of Sheffield and King's College, London, he followed E. H. Neville in the chair of mathematics at the University of Reading in 1954. Over 80 of his publications are listed in this book, and they range over a wide field of mathematics. His polished technique, reminiscent at times of the delicate stop-volley of a Wimbledon star, has helped him to clarify and to solve many problems; but his fundamental contributions to modern combinatorial theory, particularly in the establishment of the partition calculus, may perhaps be regarded as his most important and influential work.

The individual items deal with researches at a high level, connected as a rule with some aspect of Rado's own studies. One unusual paper is by van der Waerden; it describes an after-lunch session in which he, Artin and Schreier together arrive at a proof of Baudet's conjecture that, if the positive integers are divided into two classes, at least one of the classes will contain an arithmetic progression of arbitrary length. The literature of the psychology of mathe-

matical invention is less plentiful than one would like; mathematicians seldom have time or opportunity to explain how they made their discoveries, even if they can remember how tentative gropings and sudden illuminations eventually developed into rigorous demonstration. Hence van der Waerden's detailed reconstruction is a most interesting and valuable complement to the writings of Hadamard and Polya.

T. A. A. BROADBENT

Cut and Glue Topology

Surgery on Compact Manifolds. By C. T. C. Wall. (London Mathematical Society Monographs, No. 1.) Pp. x+280. (Academic: London and New York, December 1970.) £5.00.

GEOMETRIC topology has come a long way from the old party trick of cutting a Möbius band down the middle and producing a connected piece of paper. This technique of cutting up surfaces and glueing them together again has been generalized to higher dimensions and is now called surgery. Wall's book is an attempt to collate all the known results of surgery (plus a few more) and bind them all in one cover. So let me start by saying that this is a very important book and should be bought by anyone at all interested in topology.

The initial idea of surgery is reasonably easy to understand. One tries to cut up and reglue a space (or more correctly a continuous function between spaces) and get something simpler. The interest lies in the case where there is an obstruction to doing this. In the simply connected case, the obstructions vanish for odd dimensional spaces and for even dimensional spaces the signature and the Arf/Kervaire invariant are obstructions. In the non-simply connected case there are obstructions which lie in the Wall group of the fundamental groups. These groups are fearfully hard to pin down and have only been calculated in a few cases.

After some preliminary results and statements in chapters 0 and 1, the author is already able by page 19 to find a counter-example to Mazur's relative non-stable neighbourhood theorem. In chapter 2, Poincaré complexes which are the algebraic analogue of a space are discussed. Then in chapter 3 there are some statements about the Wall groups with some proofs in chapter 4. In chapters 5 to 8 the book discusses the various special cases of even and odd dimensions, bounded and unbounded manifolds. In chapter 9 a proof of the principal theorem is given and some applications follow from chapter 10 onwards. These applications include manifold structures on Poincaré complexes, embeddings with submani-

folds which separate and/or have one or two sides, real fake projective spaces (surely a joke?), fake lens spaces and other goodies. At the back of the book is a notation index and very welcome it is too. Unfortunately, with all this mathematics, the material is so condensed as to be almost unreadable and some sections are particularly obscure. Indeed, one gets the impression that a lot of haste was used in the compiling of some chapters. I suspect that the labelling of theorems has been altered and some of the corresponding references by an oversight have been left unchanged. This may have been due to the fact that while the book was being prepared for publication the results of Kirby and Siebenmann which prepare the way for an attack on the purely topological case have appeared. Therefore one would like to see a follow up book in which the ideas are presented in a more accessible fashion, even if the ground covered is not so large.

ROGER FENN

Combinatorial Analysis

Graph Theory and its Applications. Edited by Bernard Harris. (Proceedings of an Advanced Seminar conducted by the Mathematics Research Center, United States Army, at the University of Wisconsin, Madison, October 13–15, 1969.) Pp. viii+262. (Academic: New York and London, September 1970.) £2.35.

WORKERS in combinatorial analysis approach their subject with an endearing exuberance, born of the excitement of a relatively new and rapidly evolving field, with many applications within as well as outside mathematics. The seminar where the papers collected in this volume were presented is described by the editor as having been "one of the most colourful events in recent mathematical history"—and so it may well have been; unfortunately the colour has all but vanished in translation to print, and the papers are, chiefly, substantial technical contributions to graph theory which will be of most interest to experts and which make little concession to the general reader. Seekers after ready applications would probably require a further expository conference!

The first essay, by F. Harary, does actually set out to relate graph theory to the social sciences, but the discussion is too slight for mathematicians and social scientists alike. There is, however, a valuable survey by V. Klee on the use of circuit codes in a typical analog-to-digital conversion system, ending with suggestions for future research; another by D. K. R. Chaudhuri on the application of graph theory to the design of experiments; and an account by R. C. Read of constructing economical algorithms which can test

given graphs for certain desired properties or construct graphs of a given kind. There is a paper by Fulkerson on blocking polyhedra, which investigates the validity of the max-flow min-cut equality and the length-width inequality in this context. An article by J. W. T. Youngs analyses the important contribution that L. Heffter made (in 1891) to the Heawood conjecture, in the light of the more recent methods which led to the proof of the conjecture in 1968 (by Ringl and Youngs). A. J. Hoffman writes about eigenvalues and colourings of graphs, J. W. Moon on mapping problems for tournaments, and W. T. Tutte generalizes to matroids a connectivity theorem of his for graphs. Finally, there are two long papers: one by R. Mullin and G.-C. Rota, in which the authors develop a systematic theory of sequences of polynomials of binomial type, of which special cases have long been in use in the calculus of finite differences, and relate it to the combinatorial theory of distribution and occupancy; and one by B. Harris and L. Schoenfeld on exponential generating functions which enumerate the number of elements in certain subsets of the symmetric semi-group on n letters.

H. HALBERSTAM

Analysis by Flame

Flame Photometry: Laboratory Practice. By J. Dvorak, I. Rubeska and Z. Rezac. English translation edited by R. E. Hester. Pp. 325. (Iliffe: London; SNTL: Prague, March 1971.) £4.50.

THE book is designed to provide a thorough introduction to the fundamental theory and practice of flame emission and absorption photometry. The emphasis, however, is placed quite firmly on flame emission spectrometry while atomic absorption spectrometry, with the exception of a description of available equipment, escapes with only a passing mention in most instances. Many of the basic concepts in flame photometry are, of course, common to both emission and atomic absorption methods of measurement, and an emphasis in this respect is quite acceptable; the reader, however, is left frequently with the impression that the book is somewhat out of date. The references cited are comprehensive up to 1967, but are apparently not sufficiently recent to have included the more significant advances made in both emission and atomic absorption spectrometry. For example, while the section dealing with various types of interferences is quite comprehensive with respect to emission measurements in relatively low temperature flames, it devotes almost no attention to the use

of the nitrous oxide-acetylene flame which overcomes some of these effects in both emission and atomic absorption spectrometry.

The book begins with a general introduction of little real value and then gives a brief but adequate description of the chief theoretical concepts of general flame photometric practice, for example, elementary theoretical relationships in emission and atomic absorption spectrometry, some flame characteristics and introduction of sample into the flame. The final and principal section of the book is concerned exclusively with practice, particularly in relation to flame emission spectrometry. Considerable attention is given to both individual instrumental aspects, such as burners, nebulizers and detectors, as well as complete instruments which were available at the time of writing. Another comprehensive section deals with interference factors likely to cause erroneous results. The final two chapters of this section deal with spectral characteristics of a range of elements and the determination of a more limited range in specific analytical applications such as water, silicates, minerals, ores and rocks, agriculture, biochemistry and so on. These chapters are of less importance and value in the light of current practice in flame photometry than probably anticipated by the authors at the time of writing. In consequence the book may be regarded as an interesting and concise, although not very thorough, introduction to flame photometric practice and is unlikely to find a widespread use among students or practising analysts.

R. M. DAGNALL

Plasma Physics

The Propagation of Electromagnetic Waves in Plasmas. By V. L. Ginzburg. Translated by J. B. Sykes and R. J. Tatler. Second edition, revised and enlarged. (International Series of Monographs in Electromagnetic Waves, Vol. 7.) Pp. xix+615. (Pergamon: Oxford and New York, January 1971.) £8.00; \$21.50.

RESEARCH in the dynamics of wave propagation in natural ionized media has increased enormously during the last decade, when results from rocket and satellite borne wave experiments have become increasingly available. This large wealth of data has stimulated the interest of many plasma physicists in the analysis and, often, in the laboratory study of a number of geophysical phenomena. For this reason alone, this new second edition of Professor Ginzburg's classic text should be a most welcome addition to the library of any plasma physicist and, in particular,

research workers in the field of ionospheric physics, as well as postgraduate students with a good grounding in basic physics.

In this new edition the presentation and the translation are substantially improved upon the old text. After reviewing the fundamental theory of electromagnetic wave propagation in a homogeneous isotropic and in a homogeneous magnetoactive plasma, the author treats propagations in inhomogeneous isotropic and inhomogeneous magnetoactive plasmas, making ample use of the methods of geometrical optics, and discussing at some length the limits of their applicability. Reflexion of radio waves from ionospheric layers is analysed in great detail. Radio wave propagation under cosmic conditions is discussed, including propagation in the solar coronal plasma and in the interstellar medium; a number of useful synoptic tables are given. The last chapter is devoted to non-linear phenomena occurring in a plasma placed in a variable electromagnetic field.

Throughout the whole book new material has been added and brought up to date. In particular, a number of non-linear phenomena have been presented in greater detail, and the discussion of pulse propagation and spatial dispersion has been greatly enlarged.

The book coverage, however, seems somewhat uneven, and a more expanded treatment would be desirable in some areas. Some topics of current interest to the magnetosphere physicist, such as wave-particle interactions, instabilities and wave generation associated with particular plasma distributions in velocity space, beam-plasma instabilities, and so on are mentioned very briefly or even totally omitted.

This apparent shortcoming may be partially due to the fact that the original Russian text of this second edition was published as long ago as 1967. This reflects in the bibliographic section, where almost 1,200 references are given, covering mainly papers up to only 1966. Of these references 383 are actually referred to in the text, while the others are intended, according to the author, to help in familiarizing the reader with recently published work. They span a period from 1958 to 1967. A minor drawback of this book, when used as a reference work, lies in the difficulty of locating some of the symbols in a non-alphabetical list given over three pages at the beginning of the text.

Like the first edition, this new edition maintains the highest standard of scholarship and exposition and, within the limitations discussed above, the author has unquestionably achieved the objective of producing a comprehensive and authoritative work.

G. MARTELLI

Arithmetic of Evolution

An Introduction to Population Genetics Theory. By James F. Crow and Motoo Kimura. Pp. xiv+591. (Harper and Row: New York and London, November 1970.) £6.05.

THE authors have been far too modest in describing this book as an "Introduction" to the theory of population genetics. They have given us a thorough review of the present state of the mathematical theory of population genetics, with references to work published as recently as 1969.

The reader will need to be familiar with genetical terms and ideas. Apart from a useful appendix on statistical and mathematical methods, few concessions are made to the reader unfamiliar with the mathematical formulation and manipulation of problems and with the interpretation of mathematical solutions. Inevitably, the mathematics is sometimes fairly advanced but numerical results and graphs are used to illustrate the conclusions. There are problems at the end of most chapters, and there is also an excellent bibliography.

The emphasis is almost entirely on evolutionary processes and the theory of natural selection. There is very little on the theory of the imposed type of selection used in animal and plant breeding programmes. The authors have deliberately avoided lengthening the book by omitting discussion of the experimental and observational data that have been used to develop and verify the theory. This decision is inevitable but sometimes creates difficulty in assessing the relative importance of different topics and in understanding exactly what it is that has to be explained or predicted.

On the technical side, I would like to have seen more use made of Markov chains and transition matrices in the discussion of finite populations and to have had some comments on the degree of relevance of results about asymptotic equilibria and stable distributions that are based on the concept of an environment constant over an infinite number of generations.

This book clearly meets the need for an advanced text. We still await more elementary texts to be used at undergraduate level for both mathematicians and biologists. Hopefully, one of these texts will emphasize breeding and selection programmes and hence the theory of artificial selection as well as natural selection.

Altogether this is an excellent book, finely produced, and is likely to be a standard work for the specialist teacher, research worker or graduate student.

R. N. CURNOW

Embryology *in vitro*

Organ Culture. Edited by J. Andre Thomas. Pp. xiii+512. (Academic: New York and London, September 1970.) £13.75.

THE present status of organ culture owes much to Etienne Wolff and his school, and this well presented book is essentially a showcase for their efforts. It is a translation of *Cultures Organotypiques* published in France in 1964, and the several authors, all colleagues of Wolff's, have taken the opportunity to update their reviews to 1969 by means of an addendum to each chapter.

After an opening discussion by Wolff on the general principles of organ culture, there are nine chapters concerning different areas of research utilizing these techniques. Differentiation of embryonic organs is the principal theme, and the clear message is that this usually occurs autonomously in accordance with normal development, whether cultured in natural or synthetic media. Within this general framework, consideration is given to interactions of cells and of tissues, the effects of hormones and inhibitors, and the culture of malignant tumours and various invertebrate tissues. There is almost no discussion on the culture of adult organs for, in Wolff's words, "These can be made to survive by means of aseptic perfusion techniques."

Not unnaturally, most of the authors are at pains to point out the value of their studies, but with the result that the text sometimes reads like a catalogue of experiments. Although styles vary, one or two chapters present a lot of information as terse statements of apparent fact, and one can only hope that these are really critical selections from the extensive literature. The addenda are obviously an easy way of updating the original articles, but there are occasions when the later information either simply confirms the previous reports or renders them redundant, except for historical interest. On the whole, however, this is an interesting and worthwhile collection.

The translators have done a good job. The text generally reads well, errors are relatively scarce, and they have even noted discrepancies in the magnification figures for some plates. The price of the book is high enough to put it beyond the reach of many aspiring, and even established, embryologists. This is a pity, for it illustrates well not only the highways and by-ways of a profitable method of research, but also the development of one man's influence on his science. We have come a long way since 1859 when the French anatomist Vulpian removed large fragments of tissue from frog embryos and "cultured" them in ordinary water.

W. D. BILLINGTON

CORRESPONDENCE

Full-time Parents

SIR,—The letter of Charles F. Louis (*Nature*, 230, 605; 1971) raises an interesting point; we have an experiment of sorts. The current generation of young adults is the first in the history of Western culture of which a substantial fraction were raised without at least one full-time parent. Is it a coincidence that this is also the first generation who in large numbers find our culture so stale, flat and unprofitable that they retire from it, who crave affection so that they indulge in "sensitivity training", "love-ins" and will copulate with anyone in sight, and who, according to some psychologists, are so insecure that they cannot be confident of their identities when not in a crowd?

Granted there are no controls, I submit that the weight of the evidence from this observation, from history and from current experiments with primates, is that children require *X* years of a full-time parent to provide guidance, protection and affection. The quality of affection may be important, but so is the quantity. To call this "sexual blackmail" is to obscure the issue. It is no more blackmail to say the price of an emotionally healthy child is five years of full-time effort by someone (mommy, daddy or nanny) than to say the price of this automobile is \$3,000. In our present culture few men would be willing to take the job, but that has nothing to do with biology. I have known a few families where it was the husband who kept house, and it seemed to work all right.

In our present situation of over-population, it would be as well if people were convinced this price exists and that they should not procreate unless they are prepared to invest enough successfully to complete the project.

Yours faithfully,

S. W. BOWNE

Department of Chemistry,
Edinboro State College,
Edinboro,
Pennsylvania 16412

Energy for Meat

SIR,—Biologist Moore's reaction (*Nature*, 230, 133; 1971) to my suggestion of tissue culture as a source of food, although intended as professional discouragement, offers no real objections to the idea. To quote the present cost per pound of culturing tissue is of course misleading—scaling up and industrializing any process reduces costs dramatically (would anyone care to buy a television set completely handmade by an electronics engineer?). The objection that the tissue growth rate is too low cannot be valid—the meat we eat at present is also grown (on the animal, as it were), and at presumably the same rate; yet we find it worthwhile waiting for it. In fact, I would assume that the different tissues of one animal have different growth rates and, for culture purposes, we would probably choose fast-growing tissues. Possibly we could accelerate the process

somehow. The question of taste is not too important; we pork and beef-eaters might find mouse tissue or compressed lymph cells of *Chlorella* objectionable, but food preference is very much a function of experience—German kids are crazy about "Salmiakpastillen", little black and salty tablets; a Chinese will smile with delight at the mention of bitter melon; and few but Americans enjoy maple syrup poured over sausages. . . .

A perhaps more serious objection, which Dr Moore missed, is the amount of energy required. In nature, the energy we get from a pound of meat ultimately derives from the Sun shining on some area where plants grow. If we culture animal (or plant) tissue in a building, we must supply the same energy in some other way. I am perhaps overly optimistic, but I believe that we are at present moving into an era of practically unlimited energy supply (what with the developments in reactor and plasma physics) and I think that our eventual problem will lie in not supply but efficiency of usage: the amount of heat dissipated into the environment by low conversion efficiency will be a limiting factor. We don't know when this point will be reached—I suspect we are a long way from it. In the meantime, let us think about food production. Some constructive thought by experts like Dr Moore is needed.

Yours faithfully,

D. BRITZ

Jülich,
Germany

Announcements

University News

The following appointments in the University of London have been announced: Dr I. Butterworth, to the chair of physics at Imperial College; Dr M. H. Lessof, to the chair of medicine at Guy's Hospital Medical School; Dr D. E. N. Davies, to the chair of electrical engineering at University College; Dr P. M. Rattansi, to the chair of history and philosophy of science at University College; Mr I. McColl, to the chair of surgery at Guy's Hospital Medical School; Dr P. J. Peterson, to the chair of botany at Westfield College; Dr C. W. Turner, to the Siemens chair of electrical engineering at King's College. The title of professor of oral immunology has been conferred on

Dr T. Lehner, in respect of his post at Guy's Hospital Medical School; that of professor of biology on Mr B. B. Boycott, in respect of his post at King's College; and that of professor of child dental health on Dr D. S. Berman, in respect of his post at the London Hospital Medical College.

Dr D. F. Jackson has been appointed professor and head of the Department of Physics in the University of Surrey.

Appointments

Dr R. H. Hedley has been appointed to the new post of deputy director of the British Museum (Natural History). Dr J. G. Sheals has been appointed keeper of zoology in succession to Dr J. P. Harding, and Dr G. B. Corbet has been appointed deputy keeper in zoology.

Miscellaneous

Sir Martin Ryle, director of the Mullard Radio Astronomy Laboratory, has been awarded the Martin N. Liebman award of the Institute of Electrical and Electronics Engineers. The Institute has presented a scholarship to Mr F. A. Huntley, University of Southampton, to visit manufacturers of semiconductors in the United States. Dr Elizabeth Laverick, technical director of Elliott Automation Radar Systems Ltd, has been elected a fellow of the Institute.

Mr S. D. Davies, technical director of Dowty Rotol Ltd, took office on May 13 as president of the Royal Aeronautical Society, in succession to Air Commodore J. R. Morgan. The following prizes were awarded at that time: George Taylor (of Australia) prize, to Professor J. H. Argyris, Imperial College; Simms prize, to K. G. Wilkinson, British European

Airways; Busk prize, to **W. J. G. Pinsker**, Royal Aircraft Establishment; Hodgson prize, to **S. D. Davies**, Dowty Rotol Ltd; Ackroyd Stuart prize, to **J. L. Edwards**, Rolls-Royce (1971) Ltd; Pilcher-Usborne prize, to **D. L. Birdsall**, University of Bristol; Alan Marsh award, to **D. A. S. Howell**, Westland Helicopters Limited.

ERRATUM. In the article "Induction of Cellular DNA Synthesis in Human Leucocytes by Epstein-Barr Virus" by P. Gerber and B. H. Hoyer (*Nature*, **231**, 46; 1971), the first sentence of the third paragraph should read "We have shown previously⁹ that leucocytes from adult human donors who had no previous EBV infection had a limited life-span *in vitro* and could not be established in long term culture".

International Meetings

June 7-9, **Cellular Antigens**, Philadelphia (Dr A. Nowotny, Temple University School of Medicine, 3400 North Broad Street, Philadelphia, Pennsylvania 19140, USA).

July 5-8, **Acetylenes, Allenes and Cumulenes**, Nottingham (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1V 0BN).

September 1-3, **Multivariable Control System Design and Applications**, Manchester (Institution of Electrical Engineers, Savoy Place, London WC2R 0BL).

September 20-October 16, **Policies for Science and Technology in Relation to Economic Development**, Brighton (The Administrator, Seminary 23, Institute of Development Studies, Andrew Cohen Building, University of Sussex, Falmer, Brighton BL1 9RE, Sussex).

September 21-24, **Quantum Theory**, York (Secretary, Department of Mathematics, University of York, Heslington, York YO1 5DD).

September 21-24, **Theory of Atomic and Molecular Spectroscopy**, York (The Secretary, Department of Mathematics, University of York, Heslington, York YO1 5DD).

September 22-23, **High Voltage Electron Microscopy**, Teddington (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

September 26-29, **Study of Lysozymes**, Herceg Novi (Professor V. Pantic, Division of Cytology and Embryology, Institute for Biological Research, 29-Novembar 142, 11000 Belgrade, Yugoslavia).

September 27-29, **Teaching Mathematics to Non-specialists**, Loughborough (Secretary and Registrar, Institute of Mathematics and its Applications, Maitland House, Warrior Square, Southend-on-Sea, Essex SS1 2JY).

September 27-October 2, **Pneumoconiosis**, Bucharest (Pneumoconiosis Conference Committee, Ministerul Muncii, Str. Scaune nr 1-3, Bucharest, Romania).

September 28-October 1, **Recent Chemistry and Biochemistry of Phenolic Compounds**, Dublin (Dr J. B. Harborne, Botany Department, The University, Reading RG1 5AQ).

September 28-29, **Protein and RNA Factors in Initiation of Protein Synthesis in Mammalian Cells**, Coventry (Dr D. W. Hutchinson, School of Molecular Sciences, University of Warwick, Coventry CV4 7AL).

October 1-5, **Basic Environment Problems of Man in Space**, Yerevan, USSR (Secretary, International Academy of Astronautics, 250 Rue Saint-Jacques, 75 Paris 5, France).

October 11-13, **Excitation Sources**, London (Meetings Officer, The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

October 12-16, **Communication and Transport**, Genoa (Istituto Internazionale delle Comunicazioni, 18 Viale Brigate Partigiane, 16129 Genova, Italy).

October 21-22, **Polymers in Cosmetic Formulations**, Paris (Mr Y. Tollard, d'Audiffret, Laboratoire de Recherches Unilever, 8, Impasse de la Montjoie, 93-La Plaine-St Denis, France).

October 25-28, **International Research and Development Exhibition and Conference**, London (G. A. Pugsley, R. W. Boardman Exhibitions Limited, 8 Leicester Square, London WC2H 7BN).

October 27-30, **Society for Neuroscience Meeting**, Washington DC (Neuroscience Meeting Headquarters, 1629 K Street NW, Suite 700, Washington DC 20006, USA).

November 5-7, **Genesis of Base Metal Deposits in Ireland**, Galway (Professor D. Skevington, Geology Department, University College, Galway, Ireland).

November 7-11, **Communication for Decision-Makers**, Denver (Miss S. Wormley, American Society for Information Science, 1140 Connecticut Avenue NW, Suite 804, Washington DC 20036, USA).

November 11, **Acoustic Surface Waves and their Applications**, London (Meetings Officer, Institute of Physics, 47 Belgrave Square, London SW1).

November 16, **Selectivity in Organic Synthesis**, London (Dr M. J. Soull, Beecham Research Laboratories, Brockham Park, Betchworth, Surrey).

November 17-19, **Rationalization of the Processing Chain**, Brussels (Dr P. A. Arias-Soto, Secretary, EVAF Textile Division, 22 Square Robert Goldschmidt, 1050 Brussels, Belgium).

November 29-December 3, **Biotelemetry**, Pretoria (Symposium Secretariat, Information and Research Services, CSIR, PO Box 395, Pretoria, South Africa).

November 29-30, **Discharge of Industrial Effluents to Municipal Sewerage Systems**, London (Mr V. H. Lewin, Institute of Water Pollution Control, Heyford Hill Cottage, Littlemore, Oxford).

December 6-11, **Exposition de Physique** France (Société Française de Physique, 33 rue Croulebarbe, Paris 13).

February 9-15, 1972, **Oceanography of the South Pacific**, Wellington (The Secretary, National Commission for Unesco, Department of Education, Private Bag, Wellington, New Zealand).

British Diary

Monday, May 24

Power Switching by Vacuum Contactors and Thyristors (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Teaching of Electrical Circuit Theory (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Tuesday, May 25

Digital Control of an Integrated Forging Press (5.30 p.m.) Mr J. P. Russell, Institution of Electrical Engineers, at Savoy Place, London WC2.

Human Conception (5.15 p.m.) Dr R. G. Edwards, Institution of Biology, with the support of the Eugenics Society, in the Lecture Hall, Natural History Museum, Cromwell Road, London SW7. (Eleventh Darwin Lecture in Human Biology.)

Printed Resistors and their Use in Precision dc Potentiometers (5.30 p.m.) Mr V. S. Umantsev, Institution of Electrical Engineers, at Savoy Place, London WC2.

RF Measurements on Solid-state Active Devices (9.30 a.m. colloquium) Institution of Electrical Engineers, jointly with the Institution of Electronic and Radio Engineers, at Savoy Place, London WC2.

Wednesday, May 26

Electricity in Horticulture (5.30 p.m.) Mr F. M. Proctor and Mr J. A. C. Weir, Institution of Electrical Engineers, at Savoy Place, London WC2.

Problems of Classification of *Haemophilus* Species (2 p.m.) Professor K. Zimmermann, University of London, at the Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12.

RF Microwave Industrial Heating (colloquium) Institution of Electrical Engineers, at the University, Bradford.

Thursday, May 27

Allergized Cells and Their Reactions (5 p.m.) Professor R. R. Coombs, at the Royal Veterinary College, Royal College Street, London NW1. (Second McFadyean Memorial Lecture.)

Annual General Meeting and Technical Films (5.30 p.m.) Institution of Electrical Engineers, at Savoy Place, London WC2.

Mechanisms 1971 (all day discussion) Institution of Mechanical Engineers, Applied Mechanics Group, at 1 Birdcage Walk, London SW1.

Rural Planning in Britain—a Study in Contrast and Conflict (5.30 p.m.) Professor G. P. Wibberley, University of London, in the Chemistry Auditorium, University College London, Gower Street, London WC1.

The Role of the Posterior Pituitary and its Hormones on the Maintenance of Conditioned Avoidance Behaviour (5.30 p.m.) Professor D. de Wied, University of London, in the Edward Lewis Lecture Theatre, The Middlesex Hospital Medical School, Mortimer Street, London W1.

Friday, May 28

Pesticides (2.30 p.m.) Mr R. J. Hance, Mr D. Lee and Mr R. J. Whiteoak, Society for Analytical Chemistry, at Fisons Ltd, Chesterford Park, Saffron Walden, Essex.

Serviceability of Multi-access Computers (5.30 p.m. colloquium) Institution of Electrical Engineers, Joint IEE/IERE Computer Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

G.R.I. Technical Report No. 8: Grass Species and Varieties: Relationships Between Stage of Growth, Yield and Forage Quality. By J. O. Green, A. J. Corral and R. A. Terry. Pp. 81. (Hurlay, Maidenhead: The Grassland Research Institute, 1971.) £1. [223]

Sick Pay Schemes. (IPM Information Report 7, New Series.) Pp. 100. (London: Institute of Personnel Management, 1971.) £1.25. [223]

Bulletin of the British Museum (Natural History). Entomology. Vol. 25, No. 7: A List of the Type-Specimens of Ephemeroptera in the British Museum (Natural History). By D. E. Kimmins. Pp. 307-324. (London: British Museum (Natural History), 1971.) 70p. [223]

Papers presented at the Conference on the Liquid Ammonia Treatment of Cellulosic Textiles, at Belle Vue, Manchester, on November 17th 1970. Pp. 46. (Didsbury, Manchester: The Cotton, Silk and Man-Made Fibres Research Association, 1971.) [223]

University of Birmingham—Research Committee. Research and Publications, 1969/1970. No. 41. Pp. 207. (Birmingham: The University, 1971.) [223]

Building Research Station Digest, No. 127: An Index of Exposure to Driving Rain. By R. E. Lacy. Pp. 8. (London: HMSO, 1971.) 5p. [223]

Ordinance Survey Annual Report 1969/1970. Pp. v+12+2 plates. (London: HMSO, 1971.) £1 net. [223]

The Carnegie Trust for the Universities of Scotland. 60th Annual Report for the year 1969/1970. Pp. 50. (Edinburgh: The Carnegie Trust for the Universities of Scotland, 1971.) [233]

Equal Pay: a Guide to the Equal Pay Act 1970. Pp. 13. (London: Department of Employment and Productivity, 1971.) [233]

A Guide to the Literature on Spectral Data. By A. Clarke. Pp. 11. (London: National Reference Library of Science and Invention, 1971.) [233]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 269, No. 1198 (18 February 1971): Electrohydrodynamic Deformation and Bursts of Liquid Drops. By S. Torza, R. G. Cox and S. G. Mason. Pp. 295-319+plates 6 and 7. (London: The Royal Society, 1971.) [233]

London School of Hygiene and Tropical Medicine (University of London), incorporating the Ross Institute and the TUC Centenary Institute. Report on the work of the School 1969/1970. Pp. 175. (London: London School of Hygiene and Tropical Medicine, 1971.) [233]

Cotton Research Corporation. Progress Reports from Experiment Stations, Season 1969/1970. Northern States, Nigeria. Pp. 35. 12p. Uganda. Pp. 67. 12p. (London: Cotton Research Corporation, 1970.) [233]

University Grants Committee Annual Survey, Academic Year 1969/1970. (Cmd. 4593.) Pp. 51. (London: HMSO, 1971.) 30p net. [233]

The Medical Research Council of Ireland. Annual Report for the year ended December 31, 1969. Pp. 73. (Dublin: Medical Research Council of Ireland, 1971.) 25p. [243]

NNL Review, Vol. 1, No. 1, January 1972. Pp. 1-36. (National Lending Library for Science and Technology.) (London: HMSO, 1971.) 50p net. [243]

Bulletin of the British Museum (Natural History). Zoology. Vol. 20, No. 8: Observations on the Systematics of Nematodes Belonging to the Genus *Syphacia* Seurat, 1916. By C. G. Oden. Pp. 253-280+5 plates. (London: British Museum (Natural History), 1971.) £1.20. [243]

Long-Distance Footpaths and Bridleways. 16-sided folder. The Countryside Commission—Descriptive Brochure. Pp. 16. (London: The Countryside Commission, 1971.) gratis. [243]

University of Oxford. Annual Report of the Curators of the Bodleian Library for 1969/1970. (Supplement No. 3 to the *University Gazette*, February 1971.) Pp. 57. (Oxford: The University, 1971.) 50p. [253]

Reading University Geological Reports, No. 4: Chemical and Mineralogical Analyses of some Basic and Ultrabasic Rocks and their Initial Weathering Products. By Andrew Parker. Pp. 7+tables and figures. (Reading: Geology Department University of Reading, 1971.) £0.50. [243]

The Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research—1970 Report. Pp. 62. (London: The Ciba Foundation, 1971.) [253]

The Physics Exhibition Handbook 1971. 55th Exhibition of Scientific Instruments and Apparatus, Alexandra Palace, London, 19/22 April 1971. Pp. xxii+330+36. (London and Bristol: The Institute of Physics, 1971.) [253]

National Council for Civil Liberties. Annual Report. Pp. 26. (London: National Council for Civil Liberties, 1971.) 15p. [263]

Information and Suggestions for the Young Academic Biologist. English Language Version: First Draft. By C. H. Waddington, assisted by Q. Siewu-Keen and Robert Wall. Pp. 62. (Edinburgh: International Union of Biological Sciences, c/o Institute of Animal Genetics, West Mains Road, 1971.) gratis. [263]

Shirley Institute. Report and Accounts, 1969/1970. Pp. 36. (Didsbury, Manchester: The Cotton, Silk and Man-Made Fibres Research Association, 1971.) [263]

Field Studies Council. Annual Report 1969/1970. Pp. 56. (London: Field Studies Council, 1971.) [263]

University of Birmingham. Annual Reports and Accounts for 1970. Pp. 48. (Birmingham: The University, 1971.) [263]

Bulletin of the British Museum (Natural History). Entomology. Vol. 25, No. 6: The Type-Material of Australasian, Oriental and Ethiopian Tachinidae (Diptera) Described by Macquart and Bigot. Pp. 251-305+1 plate. £1.80. Vol. 25, No. 8: A Catalogue of the Membracidae Types (Homoptera: Membracidae) in the British Museum (Natural History). By P. S. Broomfield. Pp. 325-386. £1.85. Vol. 25, No. 9: Gall-Forming Thrips and Allied Species (Thysanoptera: Phlaothripinae) from *Acacia* Trees in Australia. By L. A. Mound. Pp. 387-466. £2.60. Geology. Supplement 8: Middle Albian Stratigraphy in the Anglo-Paris Basin. By H. G. Owen. Pp. 164+3 plates. £6. Supplement 9: Early Tertiary Ostracoda of the Family Trachyleberididae from West Pakistan. By Q. A. Siddiqui. Pp. 98+42 plates. £8. A Handbook on Evolution. By Gavin de Beer. Fourth edition. Pp. x+130. 35p. (London: British Museum (Natural History), 1970 and 1971.) [263]

In Every Sphere. Pp. 35. (Greenford, Middx.: Tin Research Institute, 1971.) [263]

Medical Research Council. Special Report Series No. 310: Hypogammaglobulinaemia in the United Kingdom. (MRC Working Party.) Pp. xvi+319. (London: HMSO, 1971.) £4.50 net. [263]

Ministry of Agriculture, Fisheries and Food. Technical Bulletin No. 2: Laboratory Methods for Work with Plant and Soil Nematodes. Edited by J. F. Southey. Fifth edition. Pp. iv+148. (London: HMSO, 1970.) £1.15 net. [293]

Modern China Studies No. 2: Current Post-graduate Research. (International Bulletin.) Pp. vii+155. (London: Research Publications Services, Ltd., 11 Nelson Road, SE10, 1971.) [293]

Bulletin of the British Museum (Natural History). Vol. 20, No. 3: The Types and Figures Specimens of Unionacea (Mollusca: Bivalvia) in the British Museum (Natural History). By R. L. Johnson. Pp. 73-108+2 plates. (London: British Museum (Natural History), 1971.) £1.20. [293]

Anti-Locust Memoir No. 11: Outbreaks of the Australian Plague Locust (*Chortoicetes terminifera* Walk.) in New South Wales during the period 1937-1962, particularly in Relation to Rainfall. By Joyce I. Magor. Pp. 39+36 maps. (London: Ministry of Overseas Development, Anti-Locust Research Centre, 1970.) 70p. [293]

The Manufacture of Non-Ferrous Seamless Tubes: a Bibliography. Compiled by Irene A. Clarke. Second edition revised. Pp. 38. (Birmingham, Science and Technology Library, Birmingham Public Libraries, 1971.) [293]

Department of Education and Science. Statistics of Education 1969. Vol. 3: Further Education. Pp. xx+78. (London: HMSO, 1971.) £1.40 net. [293]

British Chemicals and their Manufacturers 1970: The Directory of the Chemical Industries Association Limited. Pp. xii+229. (London: Chemical Industries Association, Ltd., 1971.) gratis. [293]

Imperial Cancer Research Fund. Annual Report and Accounts 1970. Pp. 78. (London: Imperial Cancer Research Fund, 1971.) [293]

The Zoological Record. 1966, Vol. 103, Section 20: List of New Genera and Subgenera recorded in Volume 103. Compiled by H. O. Ricketts. Pp. 14. 1968, Vol. 105, Section 5: Echinodermata. Compiled by A. M. Clark. Pp. 42. £1. (London: The Zoological Society of London, 1970.) [293]

Forestry Commission. Booklet No. 32: Thinning Control in British Woodlands (Metric). By R. F. Bradley. Pp. 32. 70p net. Library Review, No. 18, December 1970. Pp. 19. Forest Record No. 71: Soil Groups of Upland Forests. By D. G. Pyatt. Pp. 51. 40p net. Bulletin No. 44: Operational Research and the Managerial Economics of Forestry. By P. A. Wardle. Pp. xxiii+140. £1.55 net. (London: HMSO, 1970 and 1971.) [303]

University of Warwick Appointments Board. Annual Report to Council for the year ended 30th September, 1970. Pp. 29. (Coventry: University of Warwick, 1971.) [303]

West of Scotland Agricultural College. Advisory Leaflet No. 86: Recommended Varieties of Herbage Plants. Pp. 5. Advisory Leaflet No. 96: Seed Mixtures for Intensive Grassland. Pp. 4. Experimental Records. No. 17: Comparison of Potentiality of Sixteen Early and Sixteen Intermediate Varieties of Perennial Ryegrass. By I. C. Hunt, J. Frame and R. D. Harkess. Pp. 35. No. 18: Comparison of Production of Sixteen Late Varieties of Perennial Ryegrass. By I. W. Hunt, J. Frame and R. D. Harkess. Pp. 22. No. 19: Comparison of Productivity from Varieties of Hybrid Ryegrass. By L. V. Hunt, J. Frame and R. D. Harkess. Pp. 25. (Auchincruive, by Ayr: West of Scotland Agricultural College, 1970 and 1971.) [303]

Hydraulic Transport of Solids in Pipes—Research and Development Facilities. Pp. 15. (Cranfield, Bedford: British Hydromechanics Research Association, 1971.) gratis. [313]

Griffin Technical Studies Brochure 1971. Pp. 40. (Alperton, Wembley: Griffin and George, Ltd., 1971.) [313]

The Sea Bird Wreck in the Irish Sea, Autumn 1969. Pp. 17. Supplement: Analytical and other Data. Edited by M. W. Holdgate. Pp. 18. Report by the Natural Environment Research Council. Edited by M. W. Holdgate. Pp. 52. (London: Natural Environment Research Council, 1971.) [313]

Office of Population Censuses and Surveys. Classifications of Occupations 1970. Pp. xxxvi+119. (London: HMSO, 1970.) £2.50 net. [383]

Hospital Research and Briefing Problems. By John Green and Raymond Moss. Additional Material by Colin Jackson. Edited by Ken Baynes. Pp. 150. (London: King Edward's Hospital Fund for London, 1971.) £1.75. [313]

Psychotherapy. By R. K. Brian. Pp. 28. (London: British Hypnotherapy Association, 1971.) 50p. [313]

University of East Anglia. Fifth Annual Report of the Appointments Board 1969/1970. Pp. 40. (Norwich: University of East Anglia, 1971.) [14]

Hill Farming Research Organization. Fifth Report 1967/1970. Pp. 104. (Edinburgh: Hill Farming Research Organization, 29 Lauder Road, 1971.) [14]

Warren Spring Laboratory. Review 1969/1970. Pp. 38. (Stevenage, Herts: Warren Spring Laboratory, 1971.) [14]

Ministry of Agriculture, Fisheries and Food—Fisheries Radiobiological Laboratory. Technical Report FRL 7: Radioactivity in Surface and Coastal Waters of the British Isles, 1969. By N. T. Mitchell. Pp. 33. (Lowestoft, Suffolk: Fisheries Radiobiological Laboratory, 1971.) [24]

Proceedings of the University of Newcastle upon Tyne Philosophical Society. Vol. 1, No. 15: Studies on the Vertebrates of Castle Eden Dene (II), a Population Study of Birds in Spring. By K. R. Ashby. Pp. 178-188. Vol. 1, No. 16: Wandering Cells in the Epidermis of the Goldfish. By Lord Richard Percy. Vol. 1, No. 17: The Occurrence of Dwarf Larvae in the Potato-root Eelworm. By Anthea M. Stephenson and Joyce Burlinson. Pp. 189-196. Vol. 1, No. 18: Re-write Rules and Transformations. By D. J. Allerton. Pp. 197-204. (Newcastle upon Tyne: University of Newcastle upon Tyne Philosophical Society, 1970.) [24]

Memoirs of the Geological Society of London. No. 5: Shallow-Water Sedimentation as Illustrated in the Upper Devonian Bagby Beds. By Roland Goldring. Pp. vii+80+12 plates. £5.25. No. 6: Late Precambrian Glaciation in Scotland. By A. M. Spencer. Pp. vii+11 plates. £5.25. (London: Geological Society of London, 1971.) [24]
PEP. Broadsheet 524: Business Education at 18+—A Survey of HND Business Studies. By W. W. Daniel. Pp. vi+91. £1. Broadsheet 525: Impact of Tax Changes on Income Distribution. By C. V. Brown. Pp. 53. £1.20. (London: PEP, 1971.) [24]

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Organometallics in Chemical Synthesis, Vol. 1, No. 1, November 1970. Published quarterly. Subscription price \$frs. 108.50 plus \$frs. 6.25 (approx. \$25.50 plus \$1.50). Pp. 1-98. (Lausanne: Elsevier Sequoia, SA, 1970.) [223]
Smithsonian Contributions to Zoology, No. 51: Studies on Ophiocomid Brittlestars. 1. A New Genus (Clarkson) of Ophiocominae with a Re-evaluation of the Genus *Ophiocoma*. By Dennis M. Devaney. Pp. 41. (Washington, DC: Smithsonian Institution Press, 1970. For sale by US Government Printing Office.) \$0.50. [223]
Institut de France. Académie des Sciences—Annuaire pour 1971. Pp. 252. (Paris: Gauthier-Villars, 1971.) [223]
Norsk Polarinstitutt. Arbok 1969. Pp. 176. (Oslo: Norsk Polarinstitutt, 1970.) [223]
Bulletin of the American Museum of Natural History, Vol. 143, Article 2: The Triassic Gliding Reptile *Icarosaurus*. By Edwin Harris Colbert. Pp. 85-142. (New York: American Museum of Natural History, 1970.) \$2.50. [223]
US Department of the Interior: Geological Survey. Water-Supply Paper 1925: Surface Water Supply of the United States 1961/1965. Part 9: Colorado River Basin. Vol. 2: Colorado River Basin from Green River to Compact Point. Pp. viii+618 (Washington, DC: Government Printing Office, 1970.) \$3.25. [223]
France: Ministère de l'Éducation Nationale. Centre National de la Recherche Scientifique. Rapport d'Activité 1968. Pp. 303. Rapport d'Activité 1969. Pp. 335. (Paris: Centre National de la Recherche Scientifique, 1969 et 1970.) [223]
Unesco. Tech. Rpt/Unesco/UNDP (SF): Tunisia—Research and Training on Irrigation with Saline Water, 1962/1969—Technical Report. Pp. iv+256. (Paris: Unesco, 1970.) [223]
Pacific Insects Monograph No. 23: Subantarctic Entomology, particularly of South Georgia and Heard Island. Edited by J. Linsley Gressitt. Pp. 374. (Honolulu, Hawaii: Entomology Department, Bernice P. Bishop Museum, 1970.) \$9.50 cloth; \$8.50 paper. [223]
Biominerallization Research Reports/Biominerallization Forschungsberichte, Band 2. Pp. 72. (Stuttgart and New York: F. K. Schattauer Verlag, 1970.) [223]
Proceedings of the North American Palaeontological Convention, Field Museum of Natural History, Chicago, September 5/7, 1969. Part E: Evolution of Communities. Pp. 409-550. \$5.25. Part F: Correlation by Fossils. Pp. 551-703. \$5.25. (Lawrence, Kansas: Allen Press, Inc., 1970.) [223]
Norsk Polarinstitutt. Meddelelser Nr. 100: The Triassic Succession of Barentsøya, Edgeøya, and Hopen (Svalbard). By B. Flood, J. Nagy and T. S. Winsnes. Pp. 20+5 plates. (Oslo: Norsk Polarinstitutt, 1971.) [223]
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Studia Forestalia Suecica, Nr. 89: New Material for Forest Yield Research: Pine, Spruce and Birch. By Manfred Näsland. Pp. 124. (Stockholm: Skogshögskolan, Royal College of Forestry, 1971.) 27 Skr. [223]
The Nucleus: Annual Review of the Science Foundation for Physics and the School of Physics within the University of Sydney, January 1971. Pp. 88. (Sydney: The University, 1971.) [223]

The Education and Training of Engineers for Environmental Health. By John Cassel *et al.* Pp. 152. (Geneva: World Health Organization; London: HMSO, 1970.) 18 Sw. francs; £1.80; \$6. [243]
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Topical-Aids: Instructional Resources for General Chemistry. By Rod O'Connor, Harry Zeitlin and Ann Zeitlin. (A Project of the Advisory Council on College Chemistry. Series 1, Publication No. 48.) Pp. iv+74. (Tucson, Arizona: Dr Rod O'Connor, Department of Chemistry, University of Arizona, 1970.) *gratis*. [243]
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Tropical Fish Culture Research Institute—Annual Report for 1969. Pp. 63. (Batu Berendam, Malacca, Malaysia: Tropical Fish Culture Research Institute, 1970.) [253]
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Gouvernement du Québec, Ministère de l'Agriculture et de la Colonisation. Recherches Agronomiques—Sommaire des Résultats 1968/1969. Pp. 141. (Québec: Ministère de l'Agriculture et de la Colonisation, 1969.) [293]
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Economic Council of Canada. Report on Intellectual and Industrial Property. Pp. x+236. (Ottawa: Information Canada, 1971.) [293]
The Experiment Station of the South African Sugar Association. Annual Report 1969/1970. Pp. 46. (Mount Edgecombe, Natal: Experiment Station of the South African Sugar Association, 1970.) [293]
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30 June-4 July 1969. Vol. 2: Summaries. Pp. 105. (Geneva: International Labour Office, 1970.) *gratis*. [293]
CERN—European Organization for Nuclear Research. Physics and Astrophysics. By E. Schatzman. (Lectures given in the Academic Training Programme of CERN.) Pp. 110. (Geneva: CERN, 1970.) [293]
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CERN—European Organization for Nuclear Research. Proceedings of the 1970 CERN Computing and Data Processing School, Varenna, Italy, 30 August-12 September, 1970. Pp. ix+469. (Geneva: CERN, 1971.) [303]
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République du Burundi. Institut des Sciences Agronomiques du Burundi. La Fumure Minérale du Caféier d'Arabie au Burundi. Par S. Coussemont, avec la collaboration de W. Gaie, F. Warnier et G. Sottiaux. Pp. 50. (Bruxelles: Institut National pour l'Étude Agronomique du Congo, 1971.) [303]
Berichte des Deutschen Wetterdienstes. Nr. 118: Beiträge zur Phänologie Europas II. Von Fritz Schnelle. Pp. 10+4 karten. 39.39 DM. Nr. 119: Geländeklimakartierung eines Messtischblattbereiches dargestellt am Beispiel der Frostgefährdung des Bereiches Ahrensburg. Von Ernst Franken. Pp. 38. Nr. 120: International Ozone Sonde Intercomparison at the Observatory Hohenpeissenberg, 9 January-5 February 1970. Von Walter Altmannspacher und Hans Ulrich Dütsch. Pp. 86. (Offenbach a.M.: Selbstverlag des Deutschen Wetterdienstes, 1970.) [313]
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